Epidemic Response Plan for Island Foxes at Channel Islands National Park

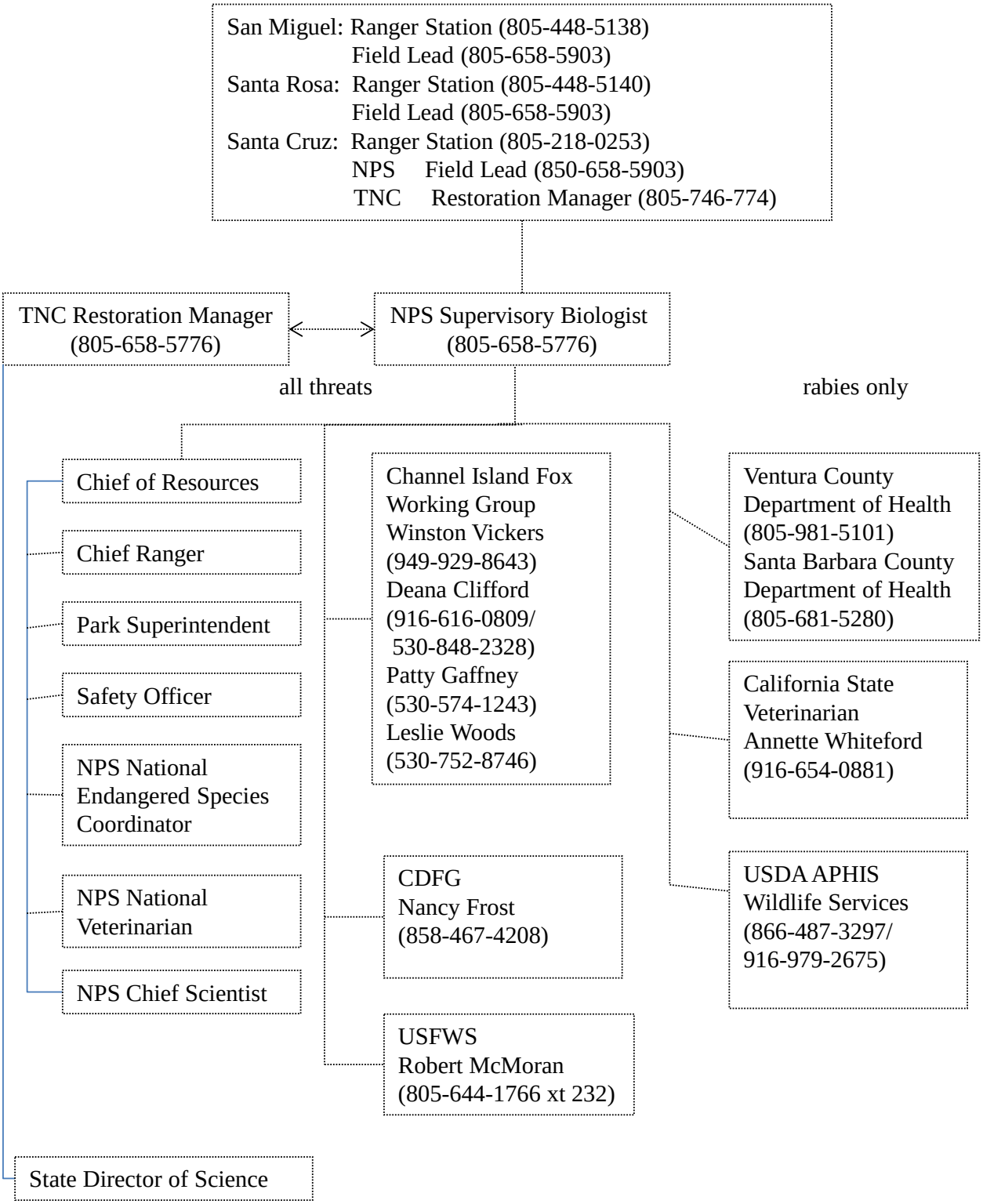
Prepared by:
The Institute for Wildlife Studies

55 Ericson Ct. Suite #1
Arcata, CA

Brian R. Hudgens
T. Winston Vickers
David K. Garcelon
Jessica N. Sanchez

November, 2013

Communication Chain in the Event of a Disease Epidemic.



Executive Summary

In recognition that island foxes (*Urocyon littoralis*) are particularly susceptible to disease epidemics, one of the recovery objectives for the listed populations of island fox is that measures for mitigating the risk of a disease outbreak have been put into place by land managers. This document provides one such measure, presenting guidelines for detecting and responding to a rapid decline in the island fox subspecies found on San Miguel, Santa Rosa, and Santa Cruz Island islands due to infectious disease or other factors. The recommendations presented here are based on consensus recommendations from a panel of experts in wildlife disease and island fox ecology, and representatives from the National Park Service (NPS) and The Nature Conservancy (TNC). This document outlines four components to mitigating the impact of a disease outbreak in island foxes: outbreak detection monitoring, a generalized response managed by an Incident Management Team (IMT), disease-specific responses, and post-epidemic monitoring.

The key to effectively responding to a disease outbreak is early detection. Early detection requires close monitoring of the fox population. An ideal monitoring program integrates observations from frequently checked radio-collared sentinel animals, long term population trends, and opportunistic behavioral observations. Observations from each monitoring technique are tied to management responses in a tiered fashion. The first responses to any suspicious observation should be to conduct a basic investigation and to notify either the NPS Field Lead or Supervisory Biologist on San Miguel and Santa Rosa Islands. On Santa Cruz Island, either the NPS Supervisory Biologist or TNC Restoration Manager should be notified. Unusual circumstances would warrant further investigation to determine if an epidemic or similar threat has been initiated within the fox population. When the available evidence suggests that foxes are in the early stages of an epidemic or facing a rapid population decline, a response is mounted characterized by three main components. First, the threat should be communicated to appropriate stakeholders. Second, incident management protocols should be initiated, including assembling IMT to hold its initial meeting. Third, the IMT should be provided with the information needed to mount a successful response. In extreme cases, preparations to trap foxes and to set up quarantine facilities should be initiated by the NPS Supervisory Biologist within 24 hours of a tier IV triggering event, even if the IMT has not yet been formed or held its first meeting.

The IMT should include the following people and representatives: Incident Commander (initially filled by the NPS Supervisory Biologist), Island Fox Conservation Group Representative, and an Island Fox Veterinary/Health Group Representative. In the case of a rabies epidemic, the IMT should also include: California State Veterinarian Representative, Ventura and Santa Barbara County Health Representatives, CA Dept. of Fish and Game Wildlife Veterinarian, and a USDA APHIS Representative.

While the IMT will be responsible for managing a disease outbreak based on ecological principles and current information about the disease course and its context, this plan includes specific recommendations for the diseases most likely to threaten island foxes. The primary response for both rabies and canine distemper will be to vaccinate healthy animals. If vaccinations prove to be ineffective or the disease has affected a large proportion of the population prior to detection, establishing a quarantined population of healthy foxes will insure against a catastrophic decline. Human health considerations will also have to be taken into account for a rabies epidemic. Establishing a quarantined population of healthy animals is the primary response to outbreaks of diseases for which no vaccine is available, such as virulent

forms of canine adenovirus or canine parvovirus. Responses to an emergent disease should include intensive monitoring to identify the disease and gather information on how it acts on and spreads between foxes. Mitigating environmental toxins that pose an acute threat to the island fox population will depend on identifying the toxin and its source.

Once a threat is reasonably considered to have passed, follow-up monitoring should continue for 1-36 months to ensure no hidden sources remain on the island. The methods and duration of post-epidemic monitoring will depend on the nature of the threat, but should include close monitoring of alternative hosts, especially the island skunk.

Contents

List of Tables

List of Figures

1. Introduction

The purpose of this document is to outline a plan for detecting and responding to a rapid decline in the island fox (*Urocyon littoralis*) subspecies found on San Miguel, Santa Rosa, and Santa Cruz Island islands due to infectious disease or other factors. Together, these islands comprise the northern Channel Island (NCI) populations of the island fox.

Island foxes are particularly susceptible to disease epidemics due to their evolutionary isolation from mainland pathogens and generally low genetic diversity (Munson 2010). The potential threat to island foxes posed by an infectious disease is highlighted by the decimation of the Santa Catalina Island fox due to a canine distemper virus (CDV) epidemic (Timm et al. 2009). Over 90% of foxes from the eastern portion of Santa Catalina Island were killed in less than two years during the epidemic, which led to the Santa Catalina Island fox being listed under the Endangered Species Act (ESA) and to an expensive ongoing recovery program (Coonan et al. 2010). The repeated introduction of raccoons to Santa Catalina Island since 1999 (Munson 2010, J King pers. comm.), coupled with the genetic similarity between the CDV strain recovered from a fox carcass and the strain typically found in raccoons, points to a stowaway raccoon as the most likely origin of the epidemic (Timm et al. 2009). There are, however, several alternative possibilities for disease introduction (Munson 2010), underscoring the possibility of an epidemic in any of the island fox populations.

The NCI fox populations share many of the same risk factors as the Santa Catalina Island fox population. The proximity of the islands to the southern California mainland facilitates exposure to potential disease vectors such as vagrant or migratory bats, or diseased animals stowed away on vessels landing on or passing near the island's shores. Because NCI foxes have limited genetic variability (Aguilar et al. 2004), populations would be highly vulnerable to severe population reduction and possibly even extinction from the introduction of a virulent infectious disease. As devastating as the CDV epidemic was to the Santa Catalina Island fox population, it was mitigated by a dispersal barrier created by a narrow isthmus and the town of Two Harbors (Coonan et al. 2010). Because there are no such barriers on the NCI, the fate of the NCI fox population in the face of an epidemic or similar threat is even more dependent on rapid and successful abatement through human management.

This document covers numerous potential threats to island foxes, but because of the risk factors identified above, it focuses primarily on the threat of a virulent epidemic disease. In order to facilitate its use by a wide audience descriptions of abbreviations and technical terms are presented in Section 2. Section 3 outlines the methods used to design this plan.

An epidemic occurs when disease is spread from an infected individual animal to one or more uninfected animals leading to high infection rates. The self-propagating nature of an epidemic has several implications for management. First, the final impact of an epidemic on foxes will likely depend on how quickly the disease is detected following initial infections. For that reason, Section 4 of this document presents monitoring strategies aimed at early detection of disease to avoid an epidemic, and Section 5 presents a tiered set of Initial Management Responses (IMRs) triggered by monitoring results. Second, the most effective management strategy will depend on complex interactions between the pathogen (disease agent), fox hosts, alternative hosts, vector species, and environmental stressors. Consequently, the ultimate success at controlling or preventing an epidemic will depend on an adaptive response by a well-informed Incident Management Team (IMT). Section 5.3 of this document outlines the

composition and roles of such a team. Third, effective management will depend on the specific pathogen involved and tools available to manage both the pathogen and its potential hosts and vectors. Section 6 outlines a general set of management actions and strategies for managing the most likely diseases to infect NCI foxes, as well as strategies for emergent diseases. Finally, effective management responses to certain pathogens will likely span several months or even years. Monitoring strategies for informing adaptive management actions and determining when a pathogen has been extirpated from the island are included in Section 6 and post-epidemic monitoring is addressed in Section 7. Although host-pathogen dynamics are often influenced by evolutionary changes in pathogen virulence (O'Keefe and Antonovics 2002) and host immunity (Van Riper et al. 1986, Woodworth et al. 2005), we do not address the potential influence of pathogen or fox evolution over the course of an epidemic because the threats addressed here are likely to endanger the NCI fox population in a short time period relative to evolutionary processes (Ebert and Bull 2003, Hudgens and Garcelon 2010).

This document also outlines a strategy for dealing with non-infectious diseases which may cause a significant population decline in NCI foxes. As is the case for infectious diseases, effective management of non-infectious disease requires an adaptive response by a well-informed IMT. The general responses provided in this document, including the communication chain and composition and roles of the IMT, apply to both infectious and non-infectious diseases. This document also provides strategies for mitigating and monitoring response effectiveness specific to non-infectious disease.

2. Terminology

This document is meant for an audience with a wide range of backgrounds, including National Park Service staff, veterinarians and epidemiological experts. As such, certain terms and acronyms commonly used by some intended readers will be new to others. To facilitate a rapid review of this document, the definitions for acronyms and many wildlife disease terms used in this document are provided in the following list:

TERM:	DEFINITION
APHIS	Animal and Plant Health Inspection Service (Department of Agriculture)
CAV	Canine Adeno Virus
CDV	Canine Distemper Virus
CNS	Central Nervous System
CPV	Canine Parvovirus
disease	Collection of symptoms which negatively impact health, survivorship or fecundity.
endemic	When used with respect to a pathogen this term refers to a pathogen that has little impact on host population dynamics either because it has little impact on host fitness or it persists at stable and low infection rates.
endemic (cont.)	When used with respect to a region (e.g., island foxes are endemic to the Channel Islands), this term refers to a taxon (e.g., species, subspecies, strain) that has undergone significant evolution in the region and is known only from that region.
epidemic	An unusually high prevalence of a disease within a host population.
event/incident	A potential threat to a NCI fox population, beginning with an observation or set of observations triggering a management response and ending with the confirmation that either the observation(s) did not signal a threat or the threat has been removed due to management actions or changes in circumstances.
IC	Incident Commander
IMR	Incident Management Response
IMT	Incident Management Team
management response	A set of actions put into place by the NPS in response to a set of observations indicating a threat to a NCI fox population.
management trigger	One or more observations that leads to a management response.
MLV	Modified Live Virus

NCI	Northern Channel Islands, specifically, San Miguel, Santa Rosa and Santa Cruz Islands
NPS	National Park Service
pathogen	A biological agent that causes an infectious disease to its host.
PIC	Point Infection Control
response tier	The set of management actions associated with a given management trigger, ranging from further investigation to initiation of trapping and placing in quarantine apparently healthy animals.
run its course	The final or acceptable endpoint of an epidemic. Typically, this will be the extermination of the pathogen from the NCI fox population.
TVR	Trap Vaccinate Release
virulence	The ability of a pathogen to cause disease in the host. Pathogens that are highly virulent cause severe disease and often lead to death of the host.

3. Methods

This document is largely based on the Draft Epidemic Response Plan for the San Clemente Island Fox. That response plan was the outcome of a workshop involving epidemiologic experts from academia, government agency, and non-governmental organizations. The participants included experts in carnivore disease, island fox pathology, island fox ecology, and ecological and epidemiological modeling. The plan authors conducted preliminary consultations with the expert group to identify the threats most likely to impact island foxes and solicit ideas for how to effectively mitigate those threats. The threats identified at this stage were rabies, canine distemper virus (CDV), canine adenovirus (CAV) and canine parvovirus (CPV). These pathogens were viewed as the most likely threats because they are prevalent in southern California wild and domestic carnivores and they are known to be highly virulent in island foxes or closely related species (see Appendix A). A second panel was convened to update the monitoring and mitigation strategies outlined in the San Clemente Island plan based on the most current research and tailor recommendations for the Northern Channel Islands. The second panel was comprised of experts in carnivore disease, island fox pathology and island fox ecology, and representatives from the National Park Service and The Nature Conservancy. The panel was provided an advance draft of this plan prior to the meeting, and comments were solicited from the panel on subsequent drafts. Unless otherwise cited, strategies to detect and manage an epidemic reflect the consensus of the expert panel (Table 1). The primary differences between this Epidemic Response Plan and the San Clemente Island Epidemic Response Plan reflect the presence of island skunks (*Spilogale gracilis amphiala*) on Santa Cruz and Santa Rosa islands, differences in the agencies responsible for managing the islands, and location specific recommendations.

Table 1. Workshop participants.

Participant	Institution	Expertise
Brian Hudgens, PhD	Institute for Wildlife Studies	mediator, population modeling, island fox ecology
David Garcelon, MS	Institute for Wildlife Studies	island fox ecology
Deana Clifford, DVM, MPVM, PhD	California State Department of Fish and Game / UC Davis	island fox disease biology, epidemiology and disease risk assessment
Patricia Gaffney	University of California, Davis	island fox pathology
Tim Coonan	National Park Service	National Park Service representative, island fox ecology
Christie Boser	The Nature Conservancy	The Nature Conservancy representative, island fox ecology
Jessica Sanchez	Institute for Wildlife Studies, Humboldt State University	disease spread in island foxes

Angela Guglielmino	National Park Service	northern Channel Island fox ecology
Renae Sattler	National Park Service	northern Channel Island fox ecology
Nathan Gregory	Institute for Wildlife Studies	conservation ecology

4. Monitoring

Rapidly detecting a threat that may cause a severe decline of the island fox population requires intensive monitoring. For monitoring to be effective, results must be tied to appropriate management actions. Monitoring effort should be intensive enough, and management triggers sensitive enough, to detect an epidemic or other type of acute population decline in its earliest stages. At the same time, the optimal monitoring effort and management trigger points must also be sensitive to the costs, both in terms of resources and political goodwill, associated with responding to non-threats. One way to efficiently and effectively detect an epidemic in its earliest stages is to maintain a sufficiently intensive monitoring program linked to a tiered set of management responses in which low-cost responses have sensitive triggers and high-cost responses are only activated by strong evidence of a threat.

The most effective monitoring strategy is to conduct frequent survival checks on a large number of radio-collared foxes. Additional cues can be observed from annual mark-recapture monitoring and casual observations. In the following section we describe a recommended telemetry-based monitoring program and associated results that would trigger each tier of a 4-tiered set of IMRs (Table 2). We then describe results from annual mark-recapture and casual observations that would trigger different tiers of the IMR. The management actions associated with each response tier are described in Section 5.

4.1. Telemetry-based monitoring

Monitoring methods: The most effective way to detect an acute decline in the fox population is frequent survival monitoring of a relatively large sample of the population through radio telemetry. This monitoring would involve placing transmitters with a "mortality" sensor on sentinel foxes, and then checking the status of those foxes frequently. A minimum of 60 and ideally 100 adult foxes should be collared as sentinels (Doak et al. 2006, Hudgens et al. 2007). Increasing the number of monitored animals and frequency of monitoring will increase the power of the monitoring program to detect an epidemic or similar threat in its earliest stages, and reduce the impact of an epidemic (Doak et al. 2006). The degree to which increases in sample size and frequency facilitate successful management depends on the standing fox population size, sample size, monitoring frequency, the spatial distribution of monitored animals, background mortality rates, and interactions between these variables (Doak et al. 2006, Hudgens et al. 2007, Sanchez and Hudgens 2012). Background mortality rates of adult island foxes are expected to be relatively low (<1% per month; T. Coonan pers. comm., C. Boser pers. comm.). Mortality rates increase substantially for older adults (Hudgens et al. 2007, BRH unpublished analysis). Consequently, the most sensitive monitoring program using the fewest foxes would focus on young animals. Aging captured animals in the field is difficult and imprecise, although there is a rough correlation between molar wear and age (Hudgens et al. 2008, Hudgens and Garcelon, 2010) and animals permanently marked as pups during long-term grid trapping or other studies can be aged correctly based on their year of first capture.

Ideally, sentinel foxes should be susceptible to potential epidemic-causing pathogens and have a low risk of dying from causes that do not pose a threat to the population (e.g., senescence). The most effective disease sentinels will be animals that have not received any prophylactic vaccines (e.g., rabies and CDV). Although ideal sentinels should also have no previous exposure to naturally circulating low-virulence strains of CDV, CPV and/or CAV, it is

both impractical to test for exposure in the field and unclear whether circulating endemic strains of these viruses provide immunity against virulent strains. There may be some value in radio-collaring a small number of CDV and rabies vaccinated animals after adequate numbers of non-vaccinated sentinels have been chosen. These vaccinated foxes would serve as additional sentinels for diseases other than CDV and rabies, as well as provide information about whether the vaccines do in fact protect island foxes in the face of an epidemic.

Table 2. Incident Management Response tiers.

Tier	Trigger	Response	Description
I	Any fox mortality	Notify NPS/TNC representative and investigate circumstances surrounding death.	Section 5.1
II	Suspicious observations possibly indicating early stages of an epidemic or other population threat	Conduct a thorough and rapid investigation to evaluate observations and determine cause of suspicious observations.	Section 5.2
III	Strong indication of early stages of an epidemic or other population threat	Alert IMT and hold first IMT meeting within 72 hrs while simultaneously investigating the threat.	Section 5.3
IV	Strong indication of established epidemic disease or other widespread population threat	Activate IMT and initiate preparations to trap and quarantine within 24 hrs while investigating threat.	Section 5.4

There is a strong relationship between the potential for successful mitigation of an epidemic and the lag time between the death of a sentinel fox and initial response (Doak et al. 2006, Sanchez and Hudgens 2012). Consequently, infrequent monitoring comes with a much greater risk of an epidemic leading to a severe fox population decline. This tradeoff is exacerbated in warm or wet conditions when a fox carcass may decompose within a few days to the point where the cause of death cannot be determined from necropsy. To maximize the potential to determine the cause of death of sentinel foxes, survival checks should be conducted daily and the status of foxes with collars transmitting a mortality signal should be confirmed within 24 hours.

The most effective spatial distribution of monitored animals covers the widest extent possible of the island while emphasizing areas with either a high risk of disease entry or where a disease is likely to spread rapidly from fox to fox. Areas with the highest risk of disease entry are those frequented by boats, including Scorpion Ranch, Smugglers Cove and Prisoners Harbor on Santa Cruz Island, Bechers Bay on Santa Rosa Island, and Cuyler Harbor on San Miguel Island.

The target sensitivity of a monitoring program should balance costs against the power to detect a decline in its early stages. If every mortality of a collared animal is detected and treated as a potential signal of an epidemic, and if an effective response can be mounted within 5 days of

detecting an epidemic, Doak et al. (2006) recommend daily monitoring of at least 40 animals to prevent a CDV epidemic and at least 60 animals to prevent a rabies epidemic. A radio-collared sample of 60-100 younger animals is required to differentiate monthly mortality rates in excess of background mortality (Hudgens et al. 2007, 2008). With the range of 50-150 sentinels, more frequent monitoring can be expected to have a greater impact detecting an epidemic before it infects a large fraction of the population (Sanchez and Hudgens 2012).

Recent telemetry-based monitoring of island fox populations has varied from none on San Nicolas Island to multiple checks each week on up to 91 animals on Santa Cruz Island (Wolstenholme 2009). Most current telemetry-based monitoring is aimed at detecting golden eagle predation, with only 10-20 collared animals serving as disease sentinels and remaining collared foxes vaccinated against rabies and CDV (Coonan 2009a, 2009b, Wolstenholme 2009); although these animals do serve as sentinels for other diseases. Only the Catalina Conservancy and Navy on San Clemente Island monitor foxes for the primary purpose of detecting a disease outbreak. On Catalina Island, 45-60 collared foxes are monitored weekly, although some of these animals are also vaccinated against rabies and CDV and are monitored primarily for other purposes (King and Duncan 2009). On San Clemente Island, 100 collared foxes are monitored every 1-5 days.

Telemetry-based management triggers: A monitoring program will only be effective if monitoring results are tied to management actions. Management actions may range in intensity from initiating further investigation to initiating an emergency response. This section describes monitoring results that should trigger management actions. The recommendations that follow assume a four-tier IMR framework, with monitoring results suggesting a greater threat leading to a more intensive IMR (Table 2). The management actions associated with each response tier are described in greater detail in Section 5. Each successive response tier describes management actions that are in addition to lower response tiers; an observation triggering a tier II response should also trigger the management actions called for in a tier I response, a tier III response should also trigger management actions called for in tiers I and II, etc. Note that monitoring programs tracking greater numbers of foxes at higher frequencies allow for greater flexibility in initial response (Table 3), by virtue of greater power to both differentiate between background and unusually high mortality rates and detecting an epidemic at an earlier stage.

A tier I response should be initiated by any mortality of a radio-collared fox. A tier I response should also be initiated if signals from 5% or more of the radio-collared foxes are lost within a single day. Additionally, when the cumulative number of radio-collared foxes unaccounted for reaches or exceeds 10% of the collared population, a tier I response should be initiated.

Table 3. Expected monthly mortality rate and numbers of mortalities within a 30-day period triggering each tier of the IMR.

No. foxes monitored	expected monthly mortality[1]	tier I trigger[2]	tier II trigger[3]	tier III trigger[4]	tier IV trigger[5]
60	0-1	1	2	3	4
80	0-1	1	2	3	5
100	0-1	1	2	3	6

1. The number of mortalities expected within a 30 day period given observed survival rates from Hudgens et al. 2007 and W Andeldt pers. comm..
2. Any mortality should trigger tier I IMR.
3. Mortalities in excess of the upper 90% CI of the expected # for a random sample of monitored foxes (Hudgens et al. 2007).
4. Mortalities in excess of the upper 95% CI of the expected # for a random sample of monitored foxes (Hudgens et al. 2007).
5. Mortalities in excess of the upper 99% CI of the expected # for a random sample of monitored foxes (Hudgens et al. 2007).

A tier II response should be initiated if the number of mortalities of radio-collared foxes within a 30-day period exceeds the upper 90% confidence interval of background mortality rates (Table 3), if 2 or more mortalities within a 60-day period occur within a limited area, or if there are unusual circumstances (e.g., a fox found dead of no apparent cause) surrounding the first mortality observed within a 30-day period.

A tier III response should be initiated if the number of mortalities of radio-collared foxes within a 30-day period exceeds the upper 95% confidence interval of background mortality rates (Table 3). A tier III response should also be initiated if clinical findings or signs associated with 2 or more mortalities are suggestive of infectious disease. Such signs would include:

Excessive blood from a body orifice or nearby bloody stool
Excess saliva or other body excretions
Excessive crusty or pus-type eye or nasal discharges
Evidence of vomiting
Excessive loss of fur (on fresh carcasses)
Obviously thickened skin on nose or foot pads

A tier IV response should be initiated if the number of mortalities of radio-collared foxes within a 30-day period exceeds the upper 99% confidence interval of background mortality rates (Table 3) or if multiple mortality events occur within a limited area within 30 days. A tier IV response should also be initiated if clinical findings or signs associated with 3 or more mortalities are suggestive of infectious disease.

4.2. Long-term population monitoring

Although annual trapping is not a viable primary monitoring protocol to detect and mitigate a rapid fox population decline, results from this type of long-term monitoring may signal an epidemic. Increased mortality estimated from mark-recapture methods may signal the presence of pathogens that, by chance, have not yet affected collared animals (e.g., have not reached areas where animals have been collared), that have a long latency period, or that exert non-lethal effects but nonetheless could impact fox populations by reducing fecundity or through interactions with other diseases to increase mortality. Annual trapping may also be used to collar animals, proactively vaccinate a sub-population of animals, and collect blood samples.

Blood samples may be checked for the presence of antibodies to many pathogens circulating through the fox population, and provide a measure of historic infectious disease dynamics.

An epidemic may be signaled through a significant drop in annual estimated apparent survival from annual mark-recapture studies. The lower bound of the 95% confidence interval of annual adult apparent survival estimated from mark-recapture data of foxes captured from 2007-2009 (70%; Garcia and Associates 2009) would serve as a reasonable threshold for concern. A mark-recapture study may also signal an epidemic if the upper bound of the 95% confidence interval of the mark-recapture survival estimate is >10% below the lower bound of the 95% confidence interval for estimated annual survival of the collared population. An epidemic may also be signaled through a significant decline in pup captures although many factors, such as drought or high fox densities, can contribute to decreased pup production. Any of these results, or a significant (e.g., 50%) and unexplained drop in the estimated density of adult foxes at any annually or biannually sampled grid should trigger a tier II response (Section 5.2).

4.3. Opportunistic observations

Because island foxes are relatively unafraid of humans, opportunistic observations of fox behavior or mortality may provide the first evidence of an epidemic or threat to the fox population. Opportunistic observations may be taken by anybody observing unusual behaviors or fox mortalities. On Santa Cruz and Santa Rosa Islands, opportunistic observations of island skunk (*Spilogale gracilis amphiala*) and resident bats can also provide important early cues that diseases with potential to impact fox populations have reached the islands.

To maximize potential for early threat detection by opportunistic observations, there must be a centralized place for observations to be reported. The receiving agent should have a visible presence to all island staff and visitors. Reporting should be encouraged by educating island personnel and park visitors about fox or skunk behaviors that might signal a disease (Box 1) and that reporting a dead fox or fox expressing unusual behaviors may prevent an epidemic. The receiving agent will be most effective if they are able to integrate across monitoring programs and apply common sense to determine when combined observations from radio-

Box 1: Behaviors in live foxes that could indicate infectious disease or toxicities. Neurologic signs that would trigger a more intensive response are marked with an asterisk (*)

- Unprovoked aggressive behavior*
- Partial or total paralyzed gait*
- Animal seems drunk or dazed*
- The "CDV pose" (Figure 1)*
- Falling down*
- Turning in circles*
- Head tilt*
- Convulsing*
- Self mutilation
- Coughing
- Frequent defecation
- Vomiting

Figure 1. CDV pose demonstrated by CDV infected kit fox photo taken shortly before death. Note hunched stance, flat ear position, head down, non-alert gaze. Photo (CDFW, BLM).

collared, long-term monitoring and opportunistic observations should trigger a higher level of IMR response than would be triggered by results from any one of these taken separately.

Any fox or skunk exhibiting abnormal neurological behaviors (see Box 1), or mortality events of skunks or bats should trigger a tier II IMR. Animals exhibiting abnormal neurological behaviors should either be trapped by personnel vaccinated against rabies or euthanized by trained personnel as soon as possible. Multiple foxes observed exhibiting neurological or otherwise suspicious behaviors should trigger a tier III IMR. Trapped animals should be kept isolated in a quarantine facility until a diagnosis can be made by a trained veterinarian. Euthanasia, if necessary, should be carried out in such a way (e.g., chemical euthanasia) as to preserve brain tissue for examination during necropsy. All carcasses should be sent to a pathologist for necropsy. Care should be taken to ensure the health and safety of personnel handling or caring for these animals.

While most mortalities caused by vehicular trauma or similar fox-human interactions (e.g., entrapment) are "normal," clusters of mortalities in remote areas of the island or mortalities of young adult animals not associated with fox-human interactions should trigger a tier II IMR. Unusually high road kill rates may also signal disease; a doubling of the mean number of road kills reported over a 30-day period (averaged over the previous 12 months) should also trigger a tier II IMR.

5. Incident Management Responses

This document describes four tiers of an Incident Management Response (IMR), with higher tiers corresponding to a more intensive response and associated with a more imminent or dire threat to a NCI fox population. The responses described in this section are meant to be generally applicable, regardless of the threat to NCI foxes. However, because rabies poses a serious human health risk, the initial response to a rabies epidemic will differ in some ways from responses to other threats. When the initial response to a rabies epidemic differs, it is noted in this section. Otherwise, recommendations for mitigating the impact of specific diseases, including rabies, on a NCI fox population are described in Section 6. An overview of the IMR framework is depicted in Figure 2.

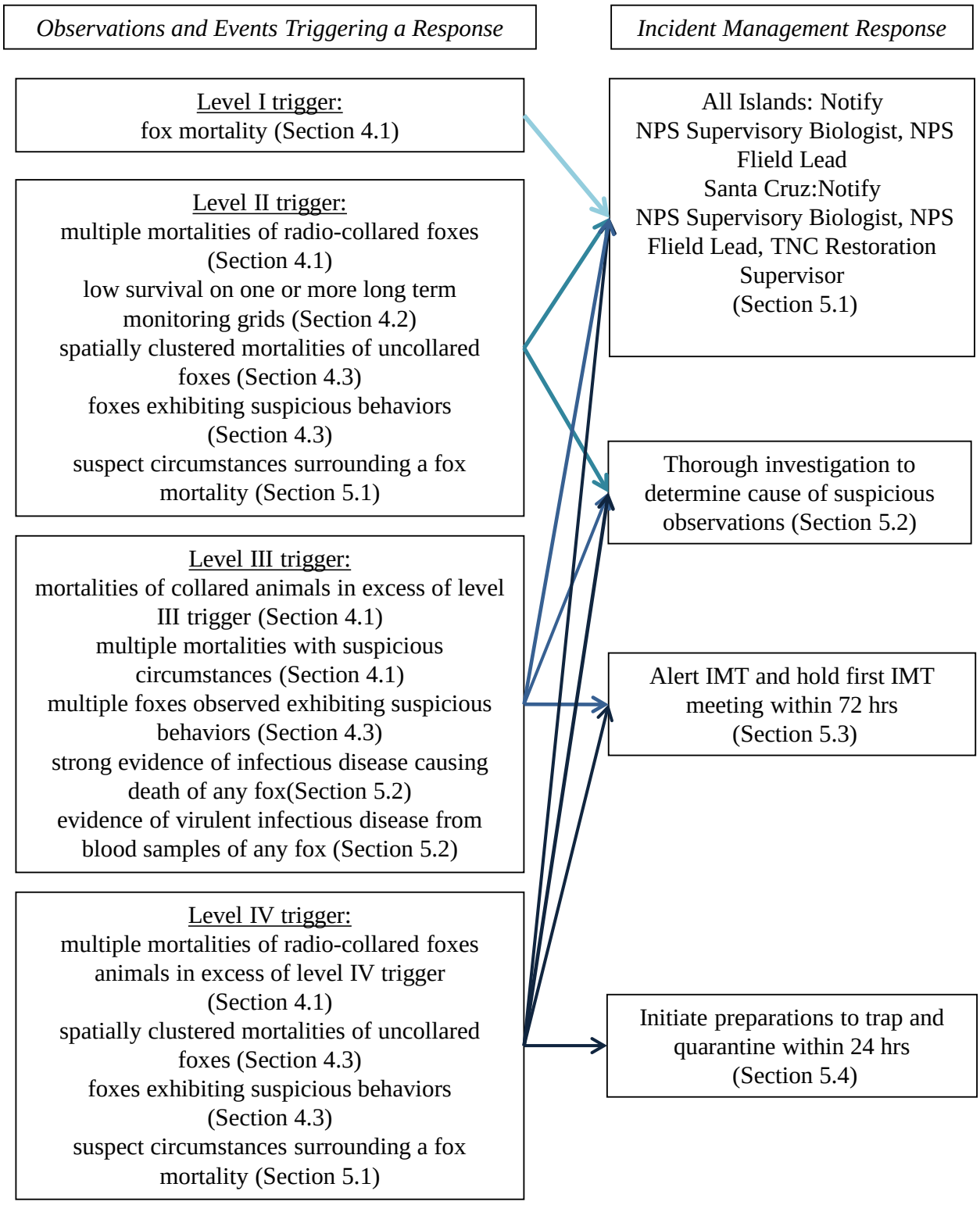
5.1. Tier I Response: Notify and Investigate

The first responses to any suspicious observation should be to conduct a basic investigation and to notify either the NPS Field Lead or Supervisory Biologist on San Miguel and Santa Rosa Islands. On Santa Cruz Island, either the NPS Supervisory Biologist or TNC Restoration Manager should be notified. In the case of a fox mortality or an unusually behaving fox, basic investigation should include a careful examination of the fox(es) involved in the suspicious incident and their immediate surroundings. At a minimum, the observer should record the following information:

- Date, time, and location of the fox
- Immediate surroundings of fox
- Status of the fox (alive or dead)
- Description of the behaviors of live foxes
- Presence of any of the following **extenuating circumstances**
  - Bloody stool or blood from any body orifice
  - Excess saliva or other body excretions
  - Crusty or pus-type eye or nasal discharges
  - Evidence of vomiting
  - Excessive loss of fur
  - Thickened skin on nose or foot pads
  - Emaciation
  - Lack of obvious cause of death for a fox carcass

If one or more extenuating circumstances are present, a tier II response should be initiated (Section 5.2). If no extenuating circumstances surround a fox carcass, it should be collected by trained personnel and stored frozen. Basic safety precautions should be taken to prevent field personnel from contracting an infection from handling a fox carcass. Field personnel should always wear gloves and dispose of or sterilize outer clothing worn while handling a carcass. Fox carcasses not sent for necropsy should be stored for at least 60 days before disposal.

Figure 2. Generalized response flowchart.



5.2. Tier II Response: Detailed Investigation

A tier II IMR is called for when there are unusual circumstances that warrant further investigation to determine if an epidemic or similar threat has been initiated within a NCI fox population. Once any of the tier II IMR criteria have been met, the first task is to inform the NPS Supervisory Biologist (on Santa Cruz, Santa Rosa, San Miguel), or TNC Restoration Manager (Santa Cruz). The second step is to identify what caused the events triggering a management response. An important key to accomplishing this task is to have a trained wildlife biologist in the field looking for additional evidence (i.e., foxes exhibiting disease symptoms or unusual behaviors, fresh fox carcasses). Locations where the fox is known to have recently visited (e.g., if the animal's location had been tracked through telemetry) should be searched for signs of environmental toxins, biological samples (feces, blood stains, other body fluids) or other evidence relevant to determining the existence, extent and underlying cause of a potential threat to the island fox population. If live trapping is being conducted, captured foxes should be swabbed for CDV and CPV (Appendix B), and protocols should be put into place to prevent disease transmission (Appendix C). Additional steps to achieve this task depend on whether the triggering event was an excessive mortality event, an observation of a live animal showing neurological symptoms, or an analysis of long-term monitoring data (including blood samples).

Mortality triggers: When multiple mortality events or extenuating circumstances surrounding a single mortality event trigger concern about a possible epidemic, carcasses of recently killed foxes should be submitted for priority necropsy. To maximize the information available from a carcass, it should be collected as soon after the animal's death as possible. Once a carcass has been collected, it should be prepared and submitted to a wildlife pathology lab for necropsy. Although there may be numerous labs capable of examining an island fox carcass, a lab with direct experience with island foxes will be best able to identify a potential threat. The labs recommended by the Island Fox Conservation Group are presented in Box 2. The first step is to contact the lab to arrange for the shipment to be received and check for special shipping instructions. Carcasses, blood, and tissue samples sent to

Box 2: Pathology Lab

The primary pathology lab handling island fox necropsies:

Dr. Leslie Woods

California Animal Health & Food Safety Laboratory

School of Veterinary Medicine

University of California

Davis, CA 95616

Phone: (530) 752-8746

Email: lwwoods@ucdavis.edu

Secondary pathology lab:

California Animal Health and Food Safety Laboratory

http://www.cahfs.ucdavis.edu/

909-383-4287

105 W Central Avenue

San Bernardino, CA 92408 2113

pathology labs should be packaged for shipping according to Department of Transportation regulations for biological specimens (U.S. Department of Transportation 2002). If rabies or other infectious disease is suspected, all material should be packaged and labeled as Biological Specimen Category A (Appendix D). Otherwise all material should be packaged and labeled as Biological Specimen Category B (Appendix D). Include a copy of all field notes and photos associated with each carcass sent for necropsy or tissue sample sent for evaluation. Arrangements should be made for necropsy results to be reported directly to the NPS Supervisory Biologist. For carcasses collected from Santa Cruz, necropsy results should also be reported to the TNC Restoration Manager.

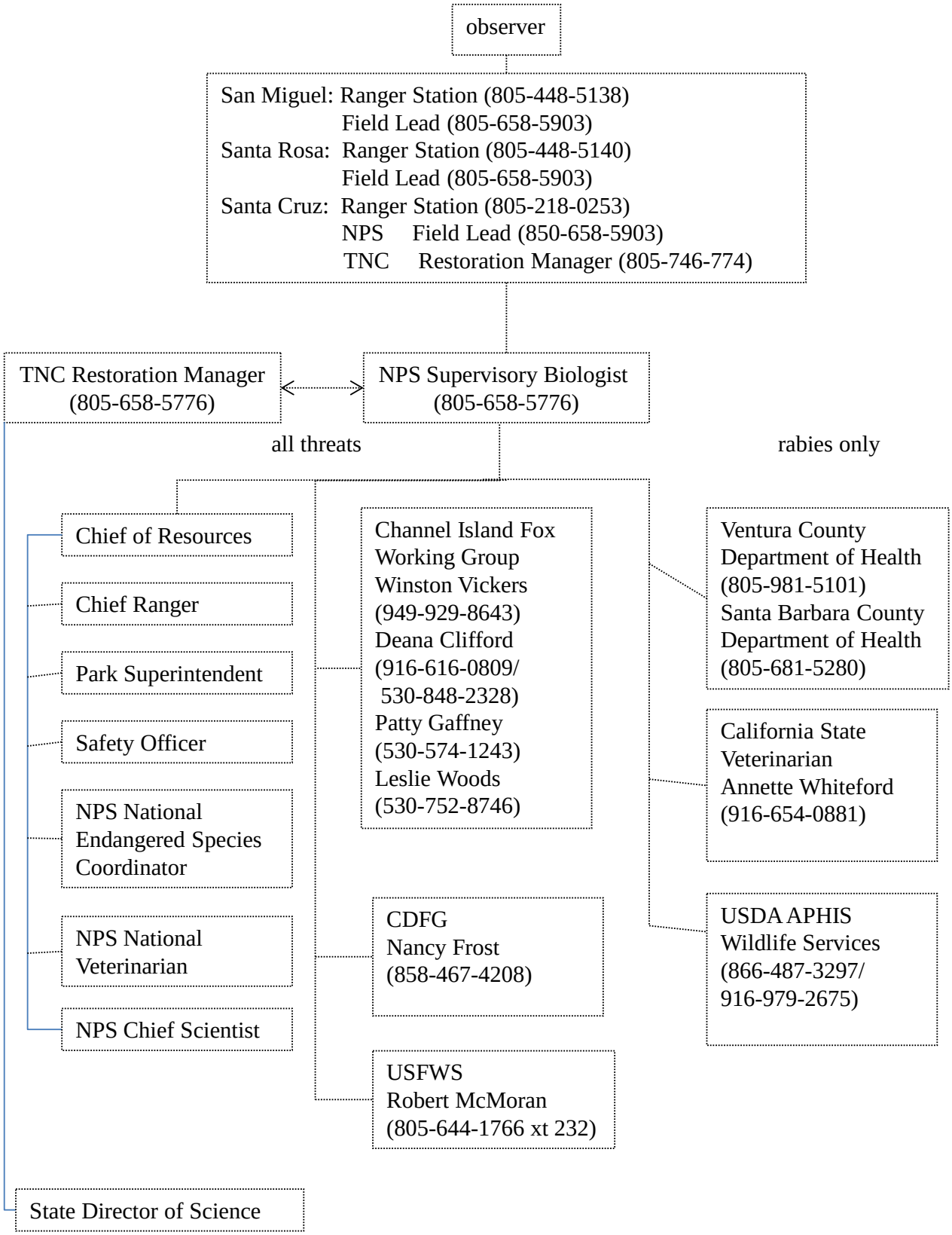
Fox behavior triggers: Because many of the pathogens posing a major risk to island foxes affect brain functions, foxes showing abnormal neurologic behaviors may be the first sign of an epidemic. The first step to responding to a neurologic fox is for personnel who have been trained and rabies-vaccinated to capture the fox if possible. If it is not possible to capture the fox due to a lack of available trained and vaccinated personnel, or if capturing the animal poses a danger to human safety (i.e., aggression by the fox), the fox should be dispatched by means of firearms. Ideally, the person(s) dispatching a suspected diseased fox should be trained in both firearm safety and wildlife biology. In order to allow an accurate diagnosis from the dispatched animal, the personnel performing this task must not damage the head and brain area.

A wildlife veterinarian/pathologist should be immediately contacted and the symptoms described. If the fox is alive, the wildlife veterinarian or pathologist may need to evaluate the animal on island. Searches for other symptomatic animals should also be initiated using trained personnel who are vaccinated against rabies. Nighttime searches by spotlight may be the most effective for observing foxes with neurologic abnormalities.

Long-term monitoring triggers: When long-term monitoring results trigger a tier II IMR, further investigation should include the following 3 steps in addition to launching a field investigation as described in Section 5.2. First, any carcasses held in storage should be sent for necropsy following the procedures outlined above for mortality triggers. Second, further data analysis and field investigation should be conducted to rule out the impacts of density dependence or an excess of old adults on estimates of survival and possibly fecundity (although no studies to date have demonstrated– or rigorously tested for– reduced fecundity in older foxes). The impact of local age structure on mortality rates could be estimated by comparing the mark-recapture estimate of apparent survival for the marked population present the previous year to what would be expected based on age-specific survival rates (e.g. Hudgens et al. 2007). Reduced survival induced by increases in lethal fox-human interactions (e.g., lower survival observed in roadside grids caused by increased road mortalities during periods of heavy deployment to the island) should also be ruled out. Third, blood samples should be collected from foxes trapped in and adjacent to the suspect long-term monitoring grid(s) and analyzed by a California state certified lab for the presence of antibodies to potential pathogens, including CDV, CAV and CPV. The Fox Veterinary Services contractor or Island Fox Veterinary/Health Group representative to the IMT (see Section 5.3) should be able to recommend an appropriate government or commercial lab.

Other investigative procedures: There are some circumstances where further investigation is warranted if necropsy results fail to support suspect diseases and/or toxins. These include a

Figure 3. Communication chain initiated by observations triggering level-III IMR.



spatial clustering of mortalities, continued high mortality rates (e.g., over two or more successive 30-day periods), or should several triggering criteria be met simultaneously without an obvious explanation. The next investigative steps depend on the surrounding circumstances. For example, spatial clustering of fox mortalities along the shoreline may implicate a marine-based toxin such as red tide (caused by the dinoflagellate *Alexandrium catenella*), a pinniped-borne disease or an offshore oil spill. Spatially clustered mortalities along a roadway or construction site might imply a toxic chemical spill. Continued high mortality during a prolonged drought period or excessively rainy year might implicate the weather. Alternatively, mortality patterns could result from a disease not detected by necropsy; emerging diseases may have no known diagnostic characteristics. If the available evidence points to no other reasonable explanation, a tier III response should be initiated and management should proceed as described for an epidemic caused by an emerging disease (Section 6.4).

5.3. Tier III Response: Initiate Incident Management Protocol

The tier III IMR is triggered when the available evidence strongly suggests that NCI foxes are in the early stages of an epidemic or facing a similar threat potentially leading to a rapid population decline. This response has three main components: communicating the threat to appropriate stakeholders, initiating incident management protocols, and providing the IMT with the information needed to mount a successful response.

Communication Chain/Notifications: In the case of a likely epidemic the communication chain should include stakeholders from the National Park Service, The Nature Conservancy, U. S. Fish and Wildlife Service, California Department of Fish and Game, the Channel Island Fox Working Group, and, in the case of rabies, the state veterinarian and Ventura and Santa Barbara Health Departmenst, and USDA APHIS. The full communication chain is presented in Figure 3.

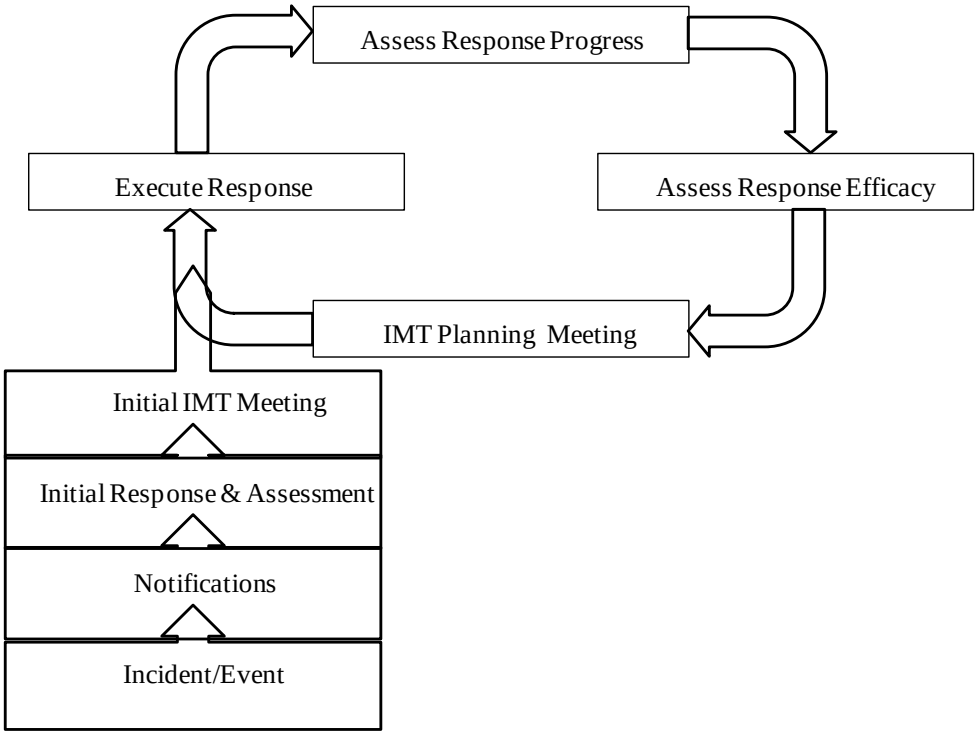
Activate incident management protocols: The generalized procedures and operational planning cycle outlined for incident management in the U. S. Coast Guard Incident Management Handbook (U.S. Coast Guard 2006) are readily adapted to the management of an epidemic or similar threat (Figure 4). The first three procedures in this process (Incident/Event, Notifications, Initial Response and Assessment) are covered in previous sections. The next step is to assemble the Incident Management Team (IMT). The IMT should include the following people and representatives:

Incident Commander
Island Fox Conservation Group Representative
Island Fox Veterinary/Health Group Representative
In the case of a rabies epidemic, the IMT should also include:
California State Veterinarian Representative
Ventura and Santa Barbara County Health Representatives
CA Dept. of Fish and Game Wildlife Veterinarian
USDA APHIS Representative

Individuals may fill multiple positions on the IMT. Initially, the NPS Supervisory Biologist will serve as the Incident Commander. The IMT should hold its initial unified command meeting within 72 hours of an event triggering a tier III response at the Incident Command Center. The preferred Incident Command Center is the Channel Island National Park headquarters in Ventura, California. Members of the IMT who cannot attend the initial meeting

in person should attend via teleconference. IMT members should be provided with a copy of this plan to review prior to the meeting. Additional copies should be maintained at Channel Islands National Park and The Nature Conservancy.

Figure 4. Operational planning cycle for threat to island fox population.



The primary purpose of the initial IMT meeting is to establish the foundation for a successful response. To meet these goals, the following tasks should be accomplished:

- Provide a brief overview of the situation
- Review the Emergency Response Plan
- Determine the positions and responsibilities of the IMT including:
  - Operations Lead
  - Planning Lead
  - Safety Lead
  - Administrative Lead
  - Finance Lead
  - Logistics Lead
- Assess equipment readiness
- Plan for initial response needs
- Identify staging areas
- Develop and implement accountability, safety and security measures
- Identify/confirm staging areas
- Determine reporting and communications
- Determine meetings schedule
- Plan for first management cycle

Management cycles are comprised of a planning meeting, executing the resulting plan, and assessing progress toward resolving the threat (Figure 4). Initial management cycles are typically completed in a 24- hour period (U.S. Coast Guard 2006), but, because most epidemics will require active management over the course of several months, management cycles may be lengthened to 7-day or 30-day cycles if deemed appropriate by the IMT.

5.4. Tier IV Response: Initiate Trap and Quarantine

In extreme cases, the difference between successfully mitigating a disease, and the severe population reduction or extinction of the NCI fox, will be highly sensitive to the time between disease detection/diagnosis and the full-scale implementation of trap and quarantine efforts. This may be the case if an infectious disease has spread to a high fraction of the fox population prior to detection, if a disease is both highly contagious and highly virulent, or for a deadly toxin that is abundant and widespread across the island. In such cases, preparations to trap foxes and to set up quarantine facilities should be initiated by the NPS Supervisory Biologist within 24 hours of a tier IV triggering event, even if the IMT has not yet been formed or held its first meeting. For tier IV events on Santa Cruz Island, the NPS Supervisory Biologist should act in coordination with the TNC Restoration Manager to establish quarantine facilities. Potential quarantine sites are shown in Figure 5, but final sites would be determined by the IMT within the first operational planning cycle. Trapping and quarantine protocols are described in Appendix C. Unless there is evidence otherwise, events triggering a tier IV response should be assumed to be caused by an emerging disease for purposes of planning (Section 6.4).

Figure 5a. Quarantine sites on San Miguel Island. Two quarantine sites are identified at historic captive breeding facility sites: Willow Canyon CBF and Brooks Canyon CBF. Quarantine sites are indicated in orange with a line connecting the label to the site location.

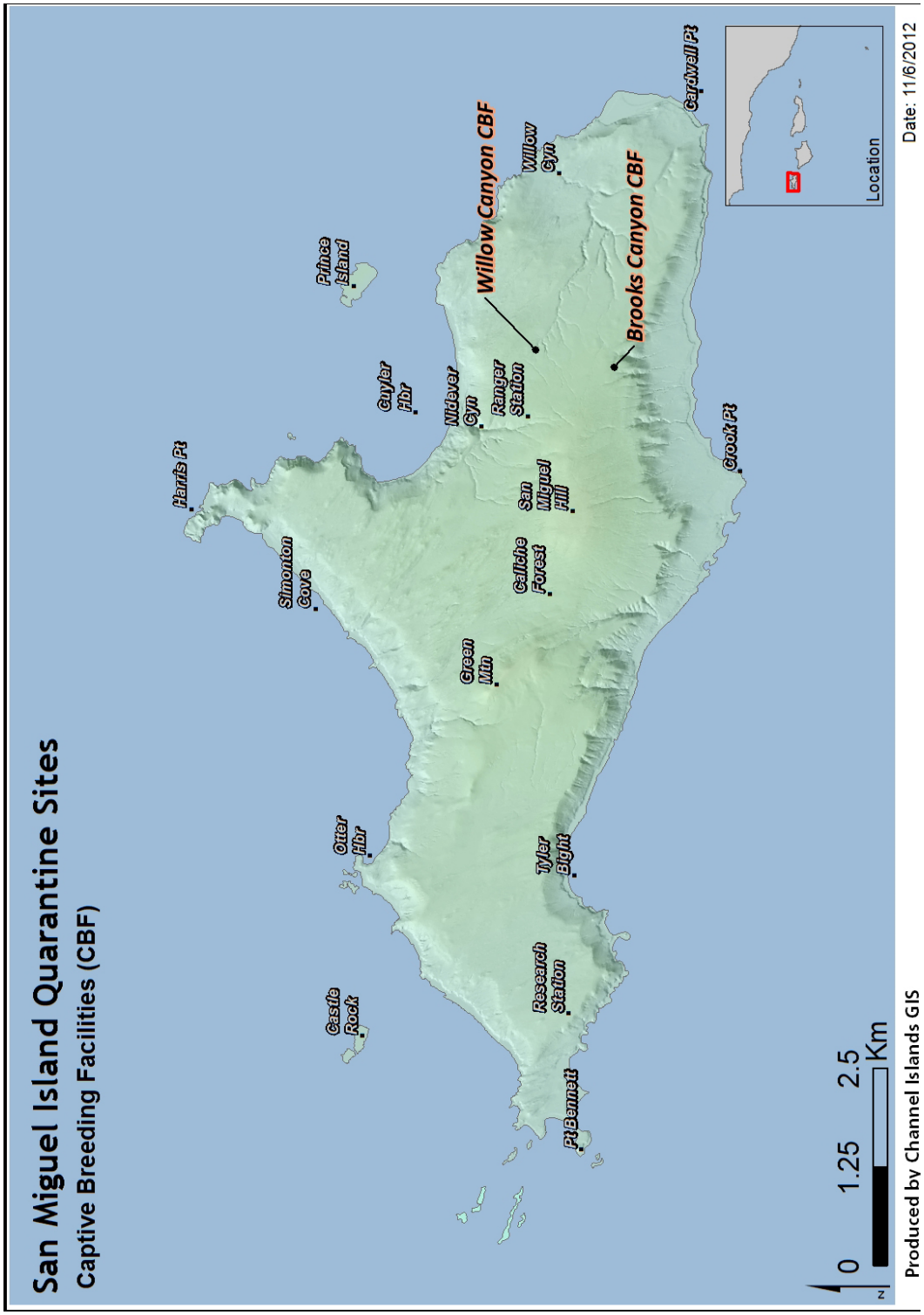


Figure 5b. Quarantine sites on Santa Rosa Island. Two quarantine sites are identified at historic captive breeding facility sites: Windmill Canyon DBF and Caballo Del Muerto CBF. Quarantine sites are indicated in orange with a line connecting the label to the site location.

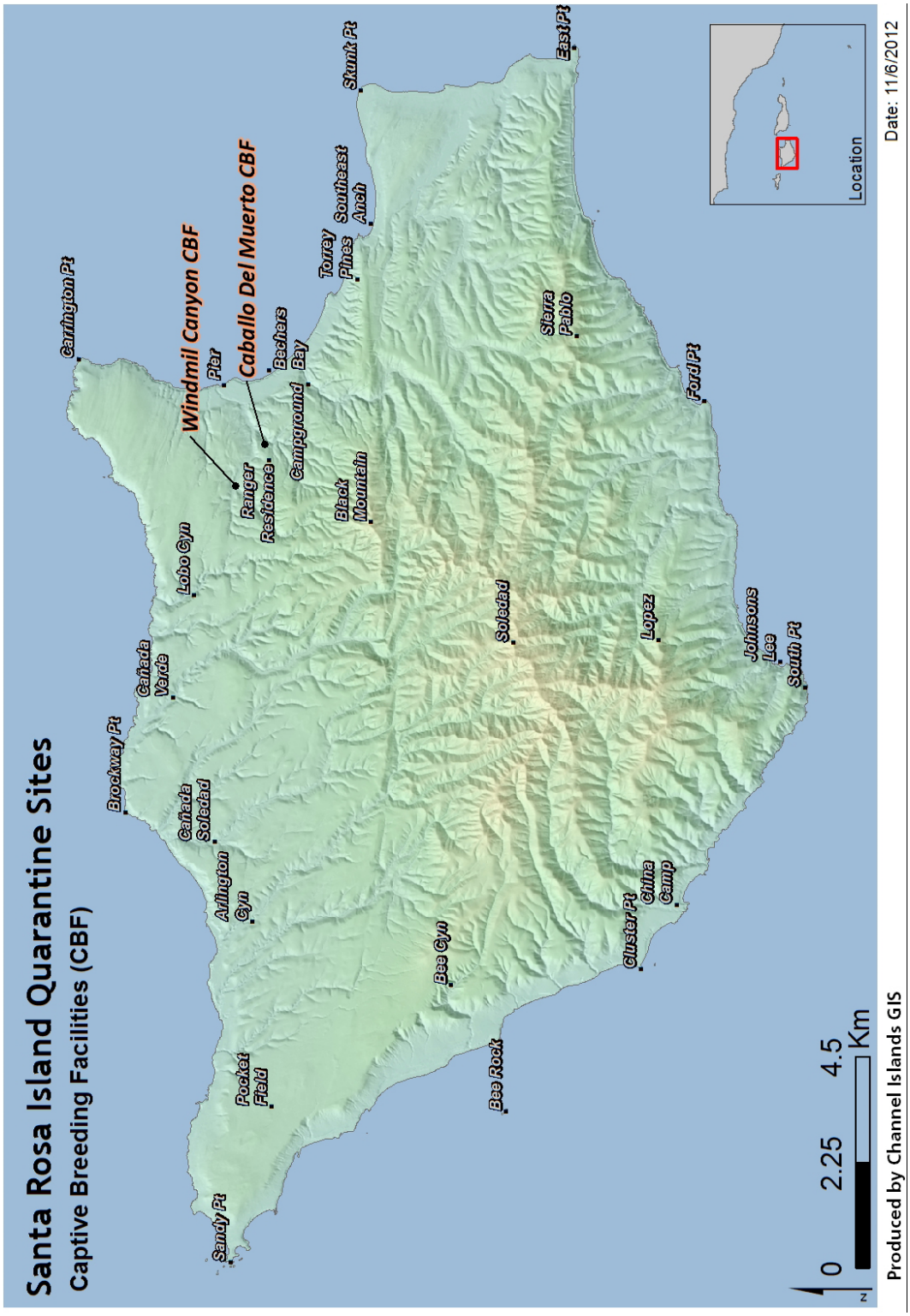
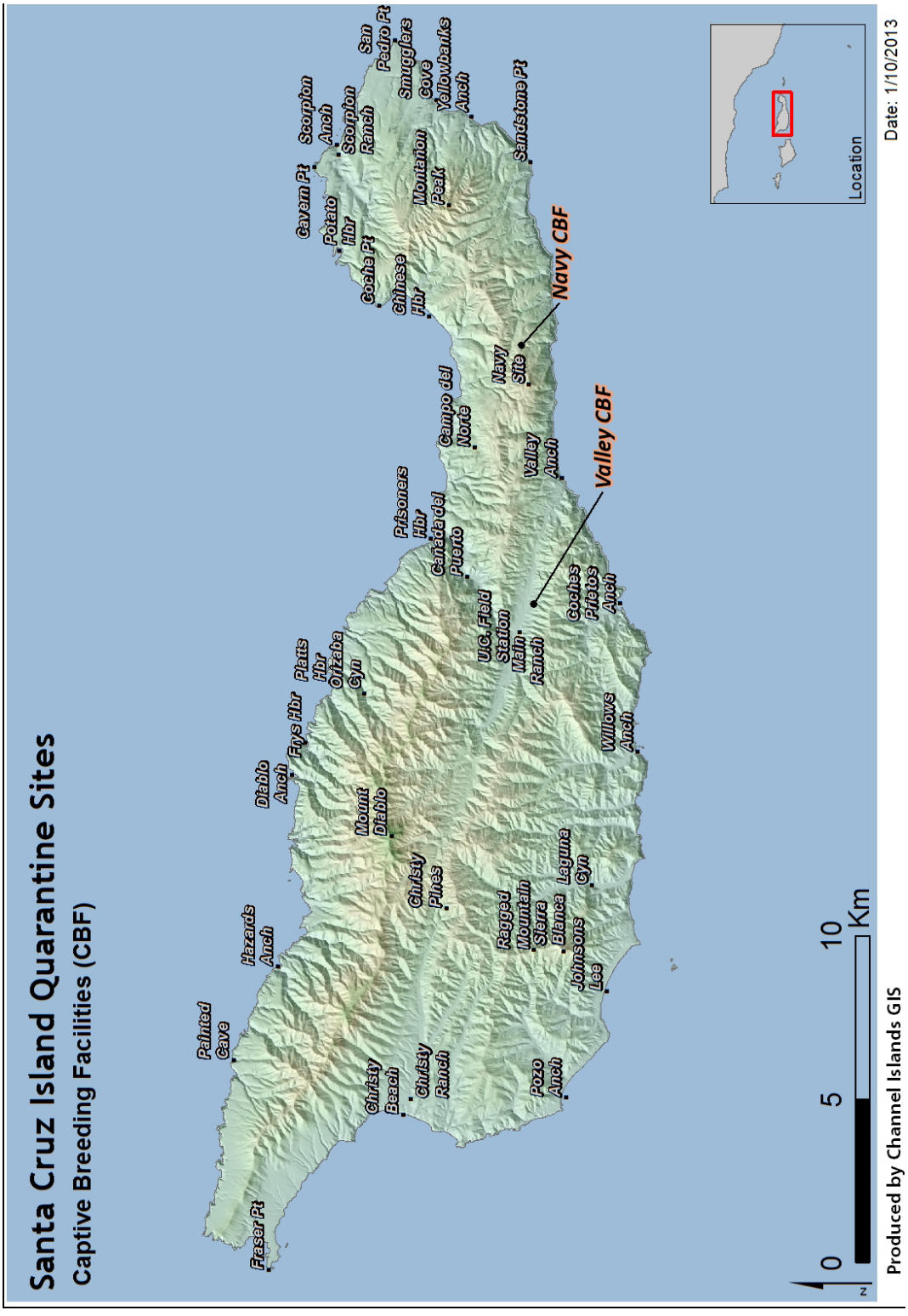


Figure 5c. Quarantine sites on Santa Cruz Island. Two quarantine sites are identified at historic captive breeding facility sites: Navy DBF and Valley CBF. Quarantine sites are indicated in orange with a line connecting the label to the site location.



6. Threat Abatement

6.1. Rabies

Rabies is a highly virulent lyssavirus primarily infecting carnivores and bats (Rupprecht et al. 2001). The primary wildlife reservoirs of rabies in the United States are raccoons (*Procyon lotor)*; skunks (*Mephitis mephitis*); bats (*Chiroptera* spp.); and red, arctic, and gray foxes (*Vulpes vulpes, Alopex lagopus, Urocyon cinereoargenteus*; Blanton et al. 2010). Cats (*Felis catus)* are the leading domestic animal in the United States diagnosed with rabies (Krebs et al. 2005) and are known to be vectors of the disease (Hanlon et al. 2007). Exposed hosts typically have a 1-3 month incubation period before becoming infectious. Rabid animals become infectious 3-10 days prior to the onset of symptoms, and almost always die within two weeks days after becoming symptomatic. Symptoms include: agitation, aggressiveness, head tilt, altered vocalizations, loss of caution, facial asymmetry restlessness, loss of appetite, vomiting or diarrhea, increased activity, increased sensitivity to stimuli, choking, drooling, drooping of the lower jaw, unequal pupil size and altered activity cycles (e.g., nocturnal animals becoming active during the day). In the final stages of the disease, symptoms typically shift to include: lethargy, frequent urination or incontinence, decreased spinal reflexes, lack of coordination, impaired movement and paralysis. While all of these symptoms are common, they may present in any combination, or not at all (Rupprecht et al. 2001). Accordingly one must be cautious and consider a rabies infection for animals with any of these presentations.

Rabies is perhaps the most problematic pathogen potentially infecting island foxes. It is highly lethal and highly contagious in foxes, readily infects sympatric island skunks, and poses a serious human health risk. Suspected rabid animals should not be approached or handled except by highly trained wildlife professionals who have been vaccinated against the disease. Because of the human health risk associated with rabies, federal, county and California state agencies *will* be involved in dealing with a rabies epidemic. Any wildlife pathologist suspecting rabies in a carcass sent for evaluation is required by law to send a sample to the California Department of Public Health or other designated lab for diagnosis. Because the state lab will discard all tissues after a diagnosis has been made, regardless of the outcome of the diagnosis, the pathologist originally receiving the carcass should send the state lab only enough brain tissue to confirm a suspected rabies case, and retain the remainder of the animal for an independent and more detailed necropsy. Details of state and county laws governing rabies can be found at http://publichealth.lacounty.gov/vet/. Because the state of California and U. S. Department of Agriculture Animal Plant Health Inspection Service (APHIS) Wildlife Services will be involved in any rabies epidemic, the National Park Service should contact the California State Veterinarian, the California Dept. of Fish and Game Wildlife Investigation Lab, and the APHIS Wildlife Services Rabies Management Program (Figure 3) before an outbreak to avoid conflicts between state and APHIS coordinated responses, and the National Park Service's management responsibilities.

The most effective way to mitigate a rabies epidemic is to rapidly vaccinate as many healthy foxes and skunks as possible, followed by monitoring to determine the effectiveness of vaccination strategies and the end of the epidemic (Figure 6). The most effective way to vaccinate a large number of animals quickly, while minimizing human exposure to the virus, is through application of baited oral vaccine. While such a vaccine is readily available and commonly used in the eastern United States, it is not currently permitted in California and can only be supplied at the request of the state veterinarian. Because the permitting process may take

an excessive period of time relative to the speed of epidemic spread on the island, a trap-vaccinate-release (TVR) protocol is presented here as the primary option, and application of baited oral vaccine as a secondary option. We do not address another strategy commonly used to manage a rabies outbreak, culling animals (see Appendix A), because the number of animals that would need to be culled in order to eliminate the disease would reduce potential host populations of foxes and skunks to small enough numbers that they would face high risk of extinction from demographic or environmental stochastic events (Smith and Wilkinson 2003);

The response to a rabies epidemic should include actions to minimize the human health risk posed by rabies. NPS staff working on the islands should be vaccinated against rabies and informed of the presence and locations of suspected rabies cases. Park campgrounds and trails within 5 km of suspected rabies cases should be closed to minimize the risk to park visitors. All visitors should be given information about the presence and extent of the epidemic and guidelines on how to identify and avoid rabid foxes and skunks. This information should be given to visitors by concessionaires providing transport to the island and through signage placed on all beaches and landing areas.

If the rabies epidemic is on Santa Cruz Island, additional considerations will need to be taken to ensure the health and safety of people outside of park service lands. The TNC Ranch manager should be vaccinated against rabies, while all other staff may be removed from the island and Island Packer Company tours on the Pelican Trail canceled. In addition the staff and visitors at the University of California field station should be informed about the presence and extent of the epidemic.

A more detailed review of rabies biology and control techniques is provided in Appendix A.

Primary Response: Trap-Vaccinate-Release: In the absence of a baited oral vaccine permitted within California, the primary response will be to trap foxes and skunks and vaccinate them using an injectable vaccine. The Imrab 3 vaccine from Merial is known to be safe for use with island foxes and is currently used to vaccinate foxes on all of the Channel Islands.

Trapping efforts should be initiated as soon as a rabies-vaccinated and highly qualified team can be assembled on island. Trapping should begin in areas located farthest away from locations of known infected animals to maximize the chance that trapped animals have not yet been exposed to the virus. Our knowledge of how quickly rabies would spread across the island is limited to infectious disease models that do not account for animal movement (Doak et al. 2006), or are based on movement and home range data from mainland fox species (e.g. Thulke et al. 2008). These models suggest that rabies is likely to spread across the island over a very short period of time. However, a spatially explicit rabies-island fox model suggests the spread may be much slower, with relatively few animals becoming infected over the course of a year (Sanchez 2012).

All asymptomatic foxes captured should be vaccinated, and permanently marked with a unique ID, such as a PIT tag. If any fox is recaptured after 2 weeks of receiving a first dose of the vaccine, it should be given a booster. After receiving its first booster, a fox should not be given another booster for 9 months. To ensure boosters are given properly, records of which animals have been vaccinated and revaccinated should be taken into the field by personnel carrying out the vaccinations. Symptomatic animals should be euthanized under the direction of a qualified veterinarian. In order to quickly eradicate the disease from the island, trapping

should continue until all foxes captured within accessible areas of the island have been vaccinated.

Island-wide TVR efforts should be repeated annually until no rabid foxes have been detected for at least 12 consecutive months. After a year has passed without an observed case of rabies on the NCI, a scaled-down vaccination program should be coupled with intensive monitoring to ensure eradication. The scaled down vaccination program would aim to deliver vaccine to a minimum of 100 adult foxes.

A sample of radio-collared foxes should be maintained and monitored as described in Section 2.2 throughout the epidemic to ensure that 1) the vaccine is effective, and 2) no other disease agents affect the population.

Secondary Response: Aerial Delivery of Baited Oral Vaccine: Aerial delivery of baited oral rabies vaccines poses the least health risk and quickest method to reduce infection rates across the island assuming island foxes consume the bait and that the ingested vaccine is effective. The baited oral vaccine RABORAL V-RG (Merial Labs) is coated with a fishmeal polymer bait containing the biomarker *tetracycline hydrochloride,* which binds to calcium in growing bone and teeth and can be used to confirm whether an animal has consumed a bait through fluorescent microscopy of thin sections of tooth or bone (Rosatte et al. 1992, Inoue et al. 2007). Baits should be shipped and stored refrigerated (2-7^{o}C), but can be kept for up to a year if stored properly.

Bait application should be geared towards vaccinating all foxes on San Miguel Island and both fox and skunk hosts on Santa Cruz and Santa Rosa Islands. An initial application should deploy at least 10 baits/host estimated to be on the island (i.e., the total numbers of foxes and skunks estimated to inhabit the island). If there is no recent population estimate, an initial application should deploy at least 5,000 baits on San Miguel Island, or 15,000 baits on Santa Cruz or Santa Rosa Islands. Bait drops should be repeated annually until no rabies cases have been observed for at least 12 consecutive months.

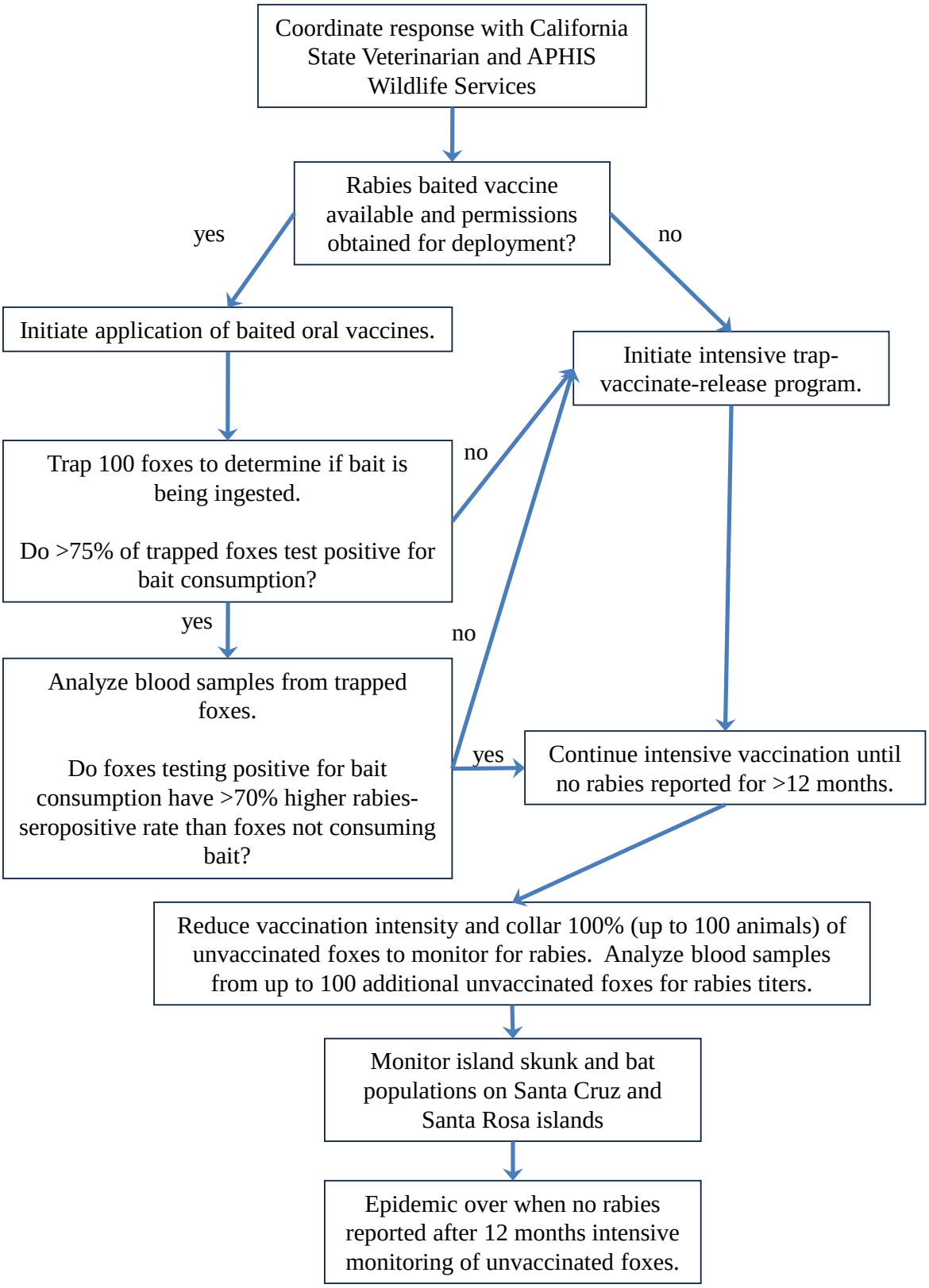
Although aerial application of oral baits has the greatest potential for mitigating a rabies epidemic, the effectiveness of this strategy must be closely monitored since it has not been previously attempted with island fox. Monitoring should focus on determining if: 1) foxes are consuming bait, 2) bait consumption leads to immunity and 3) the epidemic has run its course. The most effective way to address these questions is through a combination of repeated trapping and monitoring of radio-collared animals.

Trapping should be conducted 2-4 weeks after bait has been delivered. Captured animals should be tested for *tetracycline hydrochloride* or other bio-markers included in the bait to determine if bait is being consumed. Blood samples should be taken from apparently healthy animals and analyzed for rabies titers. In animals testing positive for bait consumption, high titers likely indicate that the vaccine has been taken up the by the animal. In animals testing negative for bait consumption, high titers indicate that the animal had been exposed to rabies prior to the time of capture. Symptomatic animals should be euthanized under the direction of a qualified veterinarian. If a high proportion (>25%) of captured foxes test negative for bait consumption, a supplemental TVR strategy should be employed (see Section 6.1.1). Determination of whether animals consuming oral vaccines are incorporating the vaccine depends on comparing exposure levels between animals that have and have not consumed the bait. If the prevalence of rabies seropositive foxes among those that have tested positive for bait consumption is not at least 70% higher than among animals testing negative for bait

consumption, a supplemental TVR strategy should be employed to achieve at least a 50% vaccination rate.

Monitoring the rabies epidemic: In order to ensure the efficacy of the epidemic response, to detect and respond to other disease agents affecting the population during a rabies epidemic, and to determine when a rabies epidemic has run its course and no longer threatens island foxes, a set of apparently healthy foxes should be radio-collared and their status monitored daily. Radio-collared foxes should include vaccinated animals throughout the course of the epidemic. Previously collared unvaccinated animals should also have their status monitored daily. The length of time for which intensive and aggressive vaccination will continue may be determined by the California Public Health Department and APHIS Wildlife Services. At a minimum, we recommend that after 12 months without a rabies case the collared animals should include up to 100 unvaccinated foxes (primarily animals born since the previous vaccination effort). In addition, blood samples from up to 100 animals that had not been vaccinated within the previous 6 months should be analyzed to determine recent exposure. On Santa Rosa and Santa Cruz Islands, a similar number of skunks should be monitored using the same protocols. Bat roosts on Santa Cruz Island should also be monitored to determine if they are harboring the disease. If no cases of rabies are detected on the island after 12 months of intensive monitoring (24 months total) the epidemic may be considered over and post-epidemic monitoring should begin (Section 7).

Figure 6. Rabies response flow diagram.



6.2. Canine Distemper(CDV)

CDV is a morbillivirus that infects members of the order Carnivora. North American hosts include coyotes, wolves (*Canis lupus*), red foxes, gray foxes, San Joaquin kit foxes (*Vulpes macrotis mutica*), black-footed ferrets (*Mustela nigripes*), striped skunks, black bears (*Ursus americanus*), and raccoons (Williams 2001). There is large variation in the susceptibility of species. For example, gray foxes are extremely susceptible and rarely survive CDV infection (Appel 1987), while red foxes show more resistance to the disease (Williams 2001). Island foxes appear to be extremely susceptible to some strains of CDV; a CDV epidemic resulted in severe population decline in Catalina Island foxes to the point where the subspecies was listed under the ESA (Timm et al. 2009). However, CDV antibodies have been observed in nearly every population of island foxes on multiple occasions (Clifford et al. 2006, Munson 2010) with no apparent effect on the fox populations. Together, these observations suggest that there are one or more endemic CDV strains circulating in island fox populations, but a CDV epidemic could be initiated by 1) an introduced virulent strain, 2) mutation of an endemic strain leading to the emergence of a virulent one, or 3) co-infection with another pathogen (Munson 2010).

Since skunks are also susceptible to canine distemper (Williams 2001), the island skunk represents a possible reservoir species for this disease. A serological survey on Santa Cruz island did not find circulating antibodies in 31 skunks tested, indicating that the native strains circulating in island foxes are not endemic in the skunks (Bakker et al. 2006).

CDV is transmitted through inhalation of aerosol droplets from the respiratory tract, or contact with bodily fluids of a sick animal (Deem et al. 2000, Williams 2001). The virus is also shed from skin, feces, and urine; however, these are considered less likely routes of transmission between individuals (Williams 2001) because the virus is generally short-lived in the environment due to its susceptibility to ultraviolet light, heat, desiccation, and disinfectants (Shen and Gorham 1980, Deem et al. 2000). The incubation period of CDV in dogs ranges from one week to over one month. Symptoms include fever, nasal discharge, conjunctivitis, anorexia, coughing, thickening of the skin on the footpads and nose, depression, vomiting, and diarrhea (Appel 1987, Williams 2001). Dogs infected with CDV suffer a 50% mortality rate (Williams 2001).

Controlling a distemper outbreak poses a number of challenges. It is highly virulent, highly contagious, and may infect a wide range of other carnivores (including cats). Vaccination is the preferred response strategy; but since there are no available oral vaccines, animals must be captured and vaccine delivered via injection. Great care must be taken not to spread the virus during the capture/vaccination process (Appendix C). Furthermore, some distemper vaccines intended for domestic dogs (modified live vaccines) have sufficient residual virulence to cause the disease in island foxes (Munson 2010). For this reason, a recombinant canarypox vectored vaccine (Merial Purevax), which has been proven safe for this species, has been the only vaccine utilized to date in island foxes. Although the vaccine is effective at eliciting an immune response in island foxes its effectiveness at conferring immunity to CDV has not been determined (Coonan et al. 2010). A final concern with vaccinating against CDV is the impact of mass vaccination on the low-virulent CDV strains endemic in the island fox population. Because these strains appear to have little negative impact on infected foxes and may confer some level of resistance against repeated epidemics, strategies to maintain endemic strains throughout vaccination campaigns should be considered. To deal with these challenges, we suggest a two-pronged approach of TVR and capture and quarantine to eradicate a virulent strain of CDV

spreading through island foxes (Figure 7). On Santa Cruz and Santa Rosa Islands, TVR efforts should include island skunks. A more detailed review of CDV biology and control techniques is provided in Appendix A.

Primary response: Trap-vaccinate-release: Once a CDV epidemic has been identified, foxes and skunks (except on San Miguel, where skunks do not occur) should be trapped and vaccinated with Merial's Purevax Ferret Distemper Virus vaccine. The modified-live canine distemper vaccine used for domestic dogs is lethal to island foxes (Coonan et al. 2010) and should not be used. Trapping should begin as far away as practical from locations of known infected animals to maximize the chance that trapped animals have not been exposed to the virus. All asymptomatic foxes and skunks captured should be vaccinated and marked with a permanent ID, such as a PIT tag. Trapping should continue until the vaccinated populations of each species reaches a total size of 300 animals or 50% of the island-wide population, whichever is greater. Higher vaccination rates may be desired if vaccine efficacy is substantially below 100%. While the vaccine may be injected subcutaneously or intramuscularly, it should be given intramuscularly in the face of an epidemic to speed absorption and antibody response. Any fox or skunk recaptured after 2 weeks of receiving a first dose of the vaccine should be given a booster. After receiving its first booster, a fox should not be given another booster for 9 months. To ensure boosters are given properly, records of which animals have been vaccinated and revaccinated should be taken into the field by personnel carrying out the vaccinations.

Because endemic strains of CDV may confer at least partial immunity to introduced virulent CDV strains, and could prevent future CDV epidemics, minimizing the extinction risk to endemic strains should be considered as part of the IMR to a CDV epidemic. One strategy to reduce the risk of extirpating endemic CDV strains while eradicating a virulent strain would be to designate a set of foxes potentially serving as hosts for the endemic strain (e.g., young of the year) to be left unvaccinated, provided that doing so does not conflict with vaccination targets for eradication of the virulent strain. If the epidemic persists for more than one year and blood tests reveal that unvaccinated animals have titers against CDV, a fraction (20-40%) of young of the year and yearling foxes should be left unvaccinated.

Because CDV may be transmitted through contact with feces or shed into the environment, precautionary steps must be taken not to spread the virus while trapping. These steps are described in detail in Appendix C.

Secondary response: Capture and quarantine: If the available vaccine does not confer immunity against the particular strain of CDV causing an epidemic, it will be necessary to capture and quarantine apparently healthy animals. Quarantine facilities should be established as soon as a CDV epidemic has been identified because the virus is likely to spread across the island before the vaccine's efficacy can be determined. A quarantine site should be identified (Figure 5), cleared of foxes and skunks and enclosed with a fox and skunk-proof fence (Appendix C) as soon as is practical. The IMT should consider the value of completing a quarantine facility and bringing foxes into captivity at the onset of a CDV epidemic in the face of uncertain vaccination efficacy and any trade-offs between doing so and maximizing resources devoted to quickly meeting vaccination targets. Methods for capturing, transporting, and housing animals in quarantine should follow the protocols outlined in Appendix C.

Monitoring: Managing a CDV epidemic will require careful monitoring to determine 1) the efficacy of vaccination, 2) the potential for exposure to low-virulence endemic CDV strains to confer immunity, and 3) when the epidemic has run its course. Monitoring should include both foxes and skunks to minimize the risk that skunks act as a reservoir allowing CDV to reenter the fox population once it has been eradicated from foxes. Monitoring should consist of daily survival monitoring of radio-collared vaccinated animals, and blood analysis of captured animals. All vaccinated animals should have blood drawn and analyzed for previous exposure to CDV. Both IGG and IGM titers should be recorded from all blood samples to assess recent exposure. For each monitored species, a minimum of 100 and up to all of the vaccinated animals should be fitted with radio collars, and any vaccinated animal found dead should be necropsied as soon as possible. Monitoring will be most effective if the monitored populations include additional radio-collared unvaccinated foxes with known exposure— both exposed and unexposed— to low-virulence endemic CDV strains. Determining the exposure status of unvaccinated foxes will not be practical in the field unless a large number of animals have had blood samples drawn and tested for CDV exposure within a year of the onset of the epidemic.

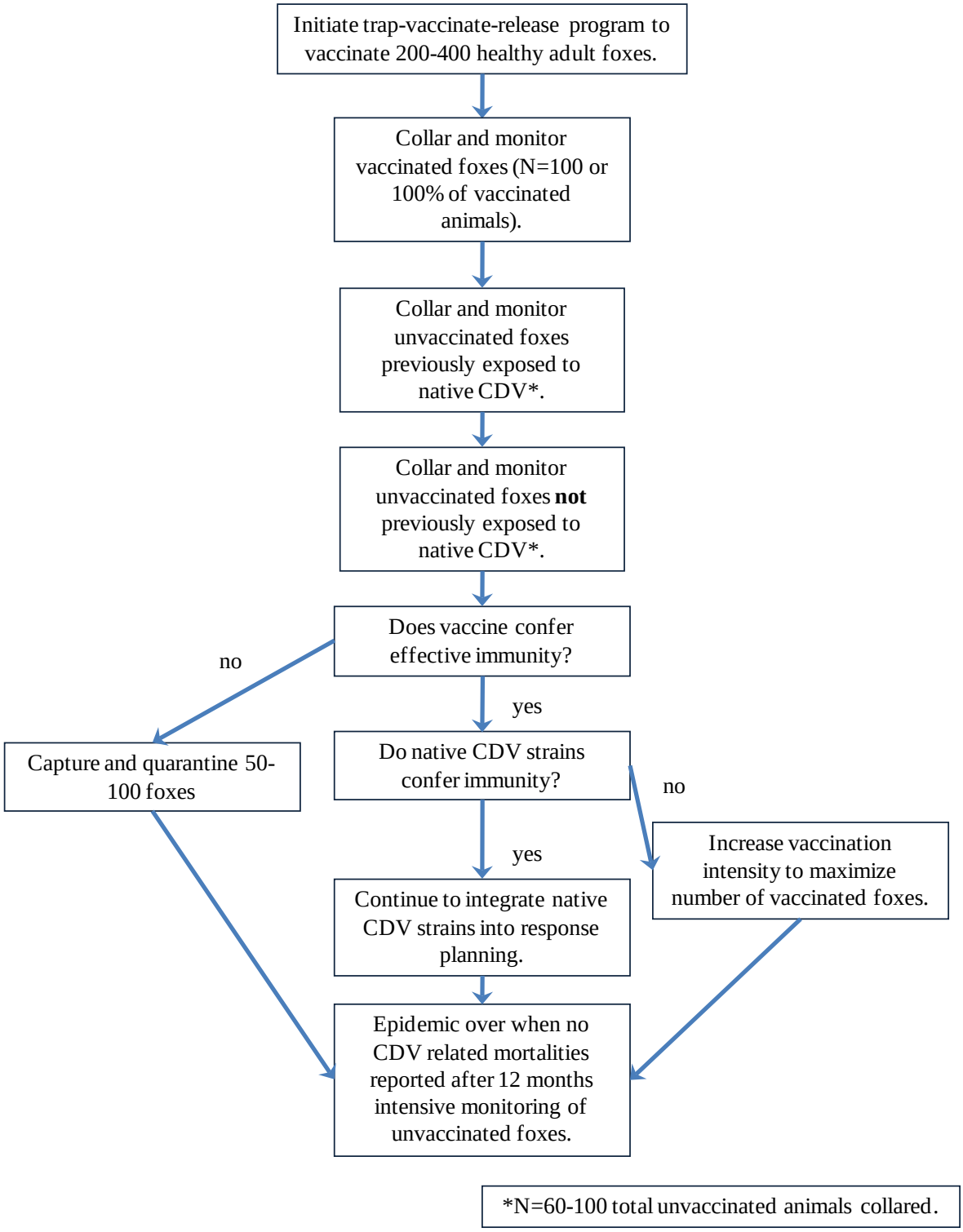
There are two monitoring results that would indicate that the vaccine has limited effectiveness against the strain causing the epidemic and that the quarantine strategy should be initiated. The first scenario would be the CDV-related death of a vaccinated fox that contracted the virus at or after the time the fox would have reached its peak immunity. Although the exact time course of a fox's immune response to the vaccine may vary from animal to animal, peak immunity should be achieved in 2-3 weeks post-vaccination (TWV unpublished data). A vaccinated fox contracting CDV would incubate the virus for 1-4 weeks before becoming symptomatic and dying. Animals dying from CDV 6-8 weeks after being vaccinated would signal that the CDV vaccine potentially has low efficacy against the particular strain driving the epidemic. In the face of a rapidly spreading epidemic coupled with low vaccine efficacy, it will be highly beneficial to begin quarantining healthy animals sooner than 6-8 weeks after most animals have been vaccinated. For that reason, we also suggest that the quarantine program be triggered if multiple vaccinated animals known not to have been exposed to CDV prior to vaccination die from CDV within 6 weeks of vaccination, or if multiple animals vaccinated in prior years (e.g., during annual preventative vaccination efforts) die from CDV within the first 8 weeks of a vaccination program. Vaccinated wild foxes should continue to be monitored in both cases to determine whether the vaccine confers some degree of immunity, and if so, the potential benefit of continuing vaccination of animals in the wild. These same recommendations applied to skunks would be prudent to the conservation of this species.

A number of scenarios would signal that endemic strains confer some immunity to the epidemic strain and the IMT should continue to incorporate maintenance of low-virulence endemic strains into the vaccination strategy. These scenarios include 1) if a high fraction (>20% higher than the highest estimated exposure rate within the previous 5 years) of CDV seropositive foxes survive for > 2 months, 2) if no unvaccinated seropositive foxes suffer CDV-related mortalities but unvaccinated seronegative foxes suffer high mortalities, or 3) if the ratio of unvaccinated seropositive:seronegative animals increases sharply during the epidemic.

After 12 months without a CDV case in either foxes or skunks, monitoring goals should shift to determine if the epidemic has ended. During this phase the collared animals should include up to 100 unvaccinated and unexposed foxes, and 100 unvaccinated and unexposed skunks on the islands where they occur. The monitored population will be primarily animals born since the previous vaccination effort. In addition, blood samples from up to 100 animals of

each species that had not been vaccinated within the previous 6 months or known to have been previously exposed to any CDV strain should be taken and analyzed to determine recent exposure. Regular spot-lighting efforts should also be made to increase the chance of detecting uncollared animals exhibiting CDV symptoms. If no cases of CDV are detected on the island after 12 months of intensive monitoring (24 months total) the epidemic may be considered to have run its course and post-epidemic monitoring should commence (Section 7).

Figure 7. CDV response flow diagram.



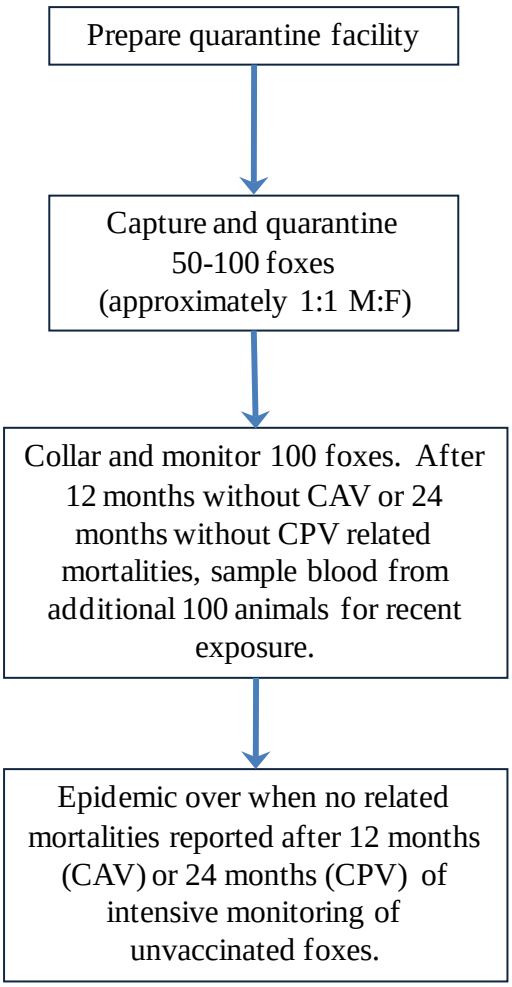
6.3. Canine parvovirus (CPV), canine adenovirus (CAV)

Canine Adenovirus and Canine Parvovirus infect canids worldwide. Both pathogens have been found in island foxes as well as coyotes, red and gray foxes and wolves (Grate et al. 1987, McCue and O'Farrell 1988, Davidson et al. 1992, Garcelon et al. 1992, Johnson et al. 1994, Acton et al. 2000, Clifford et al. 2006). CPV has also been found in island skunk (Bakker et al. 2006) and CAV is known in striped skunks (Grate et al. 1987). CPV is shed in feces (Carman and Povey 1985) and CAV is shed in urine, feces, and secretions of the eyes and nose (Woods 2001). Both pathogens are stable in the environment and may persist for months after being shed (Cabasso 1962, Pollock 1982). The incubation period for these diseases lasts for a few days followed by symptoms including anorexia, lethargy, diarrhea (usually containing blood and mucus), vomiting, dehydration, and fever for CPV infection (Montali et al. 1987, Barker and Parrish 2001) and hyperexcitability, seizures, paralysis, and coma for CAV infection (Cabasso 1981). Both diseases pose a high mortality risk to pups, although the presence of antibodies in well-monitored populations without apparently high pup mortalities suggest that there are endemic strains of both diseases circulating in island fox populations (Munson et al. 2010).

The primary challenge for dealing with CPV or CAV will be the lack of a vaccine. As indicated in Figure 8, in the absence of a working vaccine the best available strategy is to capture and quarantine a population of apparently healthy animals as described in Appendix C. This population should be maintained in quarantine until the epidemic has run its course. If the epidemic persists for multiple years, the quarantined population should be expanded after the first year with apparently healthy pups. The IMT should also consider testing on island foxes the safety and efficacy of modified live virus (MLV) vaccines used in other species (see Timm et al. 2009, Coonan et al. 2010). In extreme cases, mitigation of an extended epidemic may also require breeding captive animals, particularly since pups are especially sensitive to CPV, and CPV is capable of persisting in the environment for several months. Maintaining a breeding facility under quarantine conditions is beyond the scope of this document. Reviews of CPV and CAV biology and control techniques are provided in Appendix A.

Monitoring of wild foxes and skunks should continue to track the extent and course of the epidemic. After 24 months without a CPV case, and 12 months without a CAV case, the collared animals should include up to 100 unvaccinated foxes, and up to 100 unvaccinated skunks for epidemics on Santa Cruz and Santa Rosa Islands. In addition, blood samples should be taken from up to 100 foxes (which may include collared foxes) and analyzed to determine recent exposure to CPV/CAV. In the case of a CPV epidemic, fecal swabs should also be taken from up to 100 foxes to determine the presence of live virus through PCR (Appendix B). If no cases of CPV/CAV are detected on the island after 24 or 12 months, respectively, of intensive monitoring, the epidemic may be considered to have run its course and post-epidemic monitoring should commence (Section 7).

Figure 8. CAV/CPV response flow diagram.



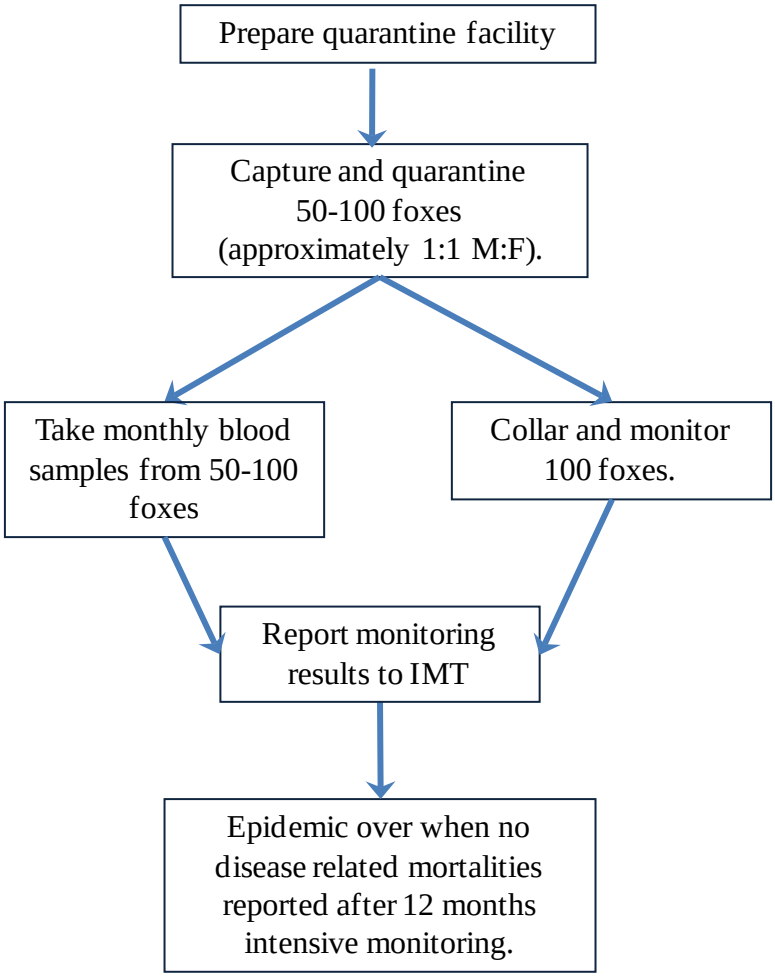
6.4. Emergent diseases

The primary challenges with an emergent disease will likely be an increased time to diagnosis, potential human health risk, and lack of a vaccine. In most cases, capture and quarantine of apparently healthy animals will be the preferred recourse to manage such an epidemic (Figure 9). The protocols for the capture, transport, and holding of animals described in Appendix C may be applied to an emergent disease. If it is determined that the disease is caused by a vector-borne pathogen (e.g., bubonic plague transmitted through fleas), vector control will be an essential tool available to control and possibly eliminate the disease from the fox population. If the emergent disease is known from and vaccinations have been developed for other species, the IMT should consider testing the safety and efficacy of those vaccinations on island foxes (see Timm et al. 2009, Coonan et al. 2010). Until it is confirmed that the emergent disease is not infectious to humans, extreme caution should be taken to prevent trapping and fox care personnel from exposure. Based on Centers for Disease Control and Prevention (CDC) safety precautions for working with hantaviruses, we advocate that workers wear a half-face air-purifying (or negative-pressure) respirator or PAPR equipped with HEPA or N-100 filters to prevent exposure from aerosolized pathogens (All About Hantaviruses…[updated 2004]). Human health precautions should be disseminated via the chain of command for other island users to take appropriate precautionary actions.

Because there will be several unknowns associated with an emergent disease, managing such an epidemic will require careful and detailed monitoring. We recommend frequent (daily or weekly) monitoring of 60-100 radio-collared animals throughout the course of the epidemic. In addition, we recommend frequent (monthly) trapping to collect blood samples from 50-100 foxes. These blood samples should be analyzed for titers or other signs of infection to 1) track infection rates, 2) determine survival of infected animals, and 3) determine the disease course within infected animals. Skunks should be monitored in the same way to determine if they serve as an alternate host. Trapping will be most effective if effort is spread across the island, and traps are set to both recapture animals trapped the previous month and capture new animals. Trapped animals displaying clinical signs of the disease should be euthanized or quarantined to monitor the disease course. All vertebrate carcasses discovered during an epidemic of an emergent disease should be necropsied as part of efforts to determine the cause and course of the disease.

The intensive monitoring called for here will help manage the epidemic by 1) providing information on the progress of the epidemic, 2) providing information that may lead to effective treatment or vaccines, 3) minimizing the threat to quarantined population posed by augmentation efforts including asymptomatic carriers, and 4) providing evidence for whether the disease is highly virulent and capable of decimating fox populations or less virulent and likely to coexist with a stable fox population, thus requiring less intensive management. Because of the uncertainty surrounding an emerging disease, it is particularly important that monitoring and interpretation of monitoring results be coordinated by personnel with expertise in wildlife epidemiology. As with the diseases discussed above, we offer the general guideline that if no cases of disease are detected on the island after 12 months of intensive monitoring the epidemic may be considered to have run its course and post-epidemic monitoring should commence (Section 7).

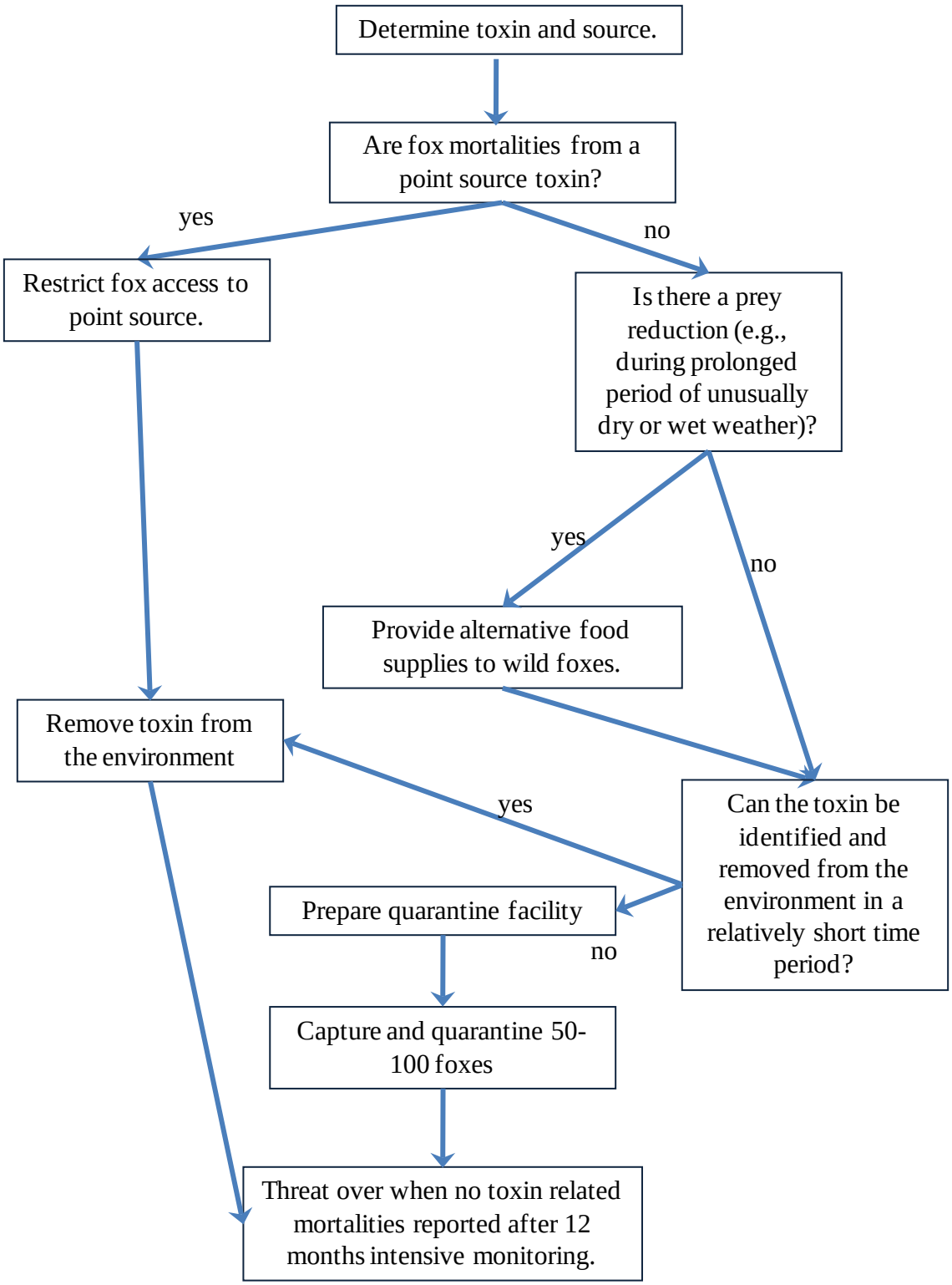
Figure 9. Emergent disesase response flow diagram.



6.5. Environmental toxin

Although environmental toxins generally are not spread animal to animal in the same way as an infectious disease, some toxins may pose an acute threat to the island fox population. Toxins may be present on the island as a result of offshore spills, chemical spills on the island, residuals from current and historic anthropogenic activities on the island, or large populations of toxin-producing organisms, such as botulism. Mitigating the effects of environmental toxins depends on identifying the toxin and its source (Figure 10). In the case of a point source (e.g., chemical spill), the threat to island foxes will be limited, and may be mitigated by a combination of restricting access to the contaminated area (e.g., surrounding the area with a fox-proof fence as described in Appendix C for the quarantine site) and proper cleanup of the contaminant. The impact of widespread toxins may be reduced by providing alternative food and water resources (e.g., spreading cat-kibble such as used to bait traps) to discourage foxes from consuming the toxins. Alternative foods will be most effective for toxins associated with prolonged spells of unusually dry or wet weather, which often reduce the availability of typical fox prey. If the source of a toxin is widespread across the island and 1) cannot be identified, 2) is highly lethal, or 3) is persistent in the environment (i.e., it will not breakdown naturally and it is not readily removed from the environment), a population of 50-100 foxes should be brought into quarantine. Survival monitoring should continue on up to 100 radio-collared animals and reproduction should be monitored by recruitment into areas trapped annually or close tracking of 30 healthy young adult (age class 1-3) foxes.

Figure 10. Environmental toxin response flow diagram.



7. Post-Threat Monitoring.

Once a threat is reasonably considered to have passed, follow-up monitoring should continue for 1-36 months to ensure no hidden sources remain on the island. The methods and duration of post-epidemic monitoring will depend on the nature of the threat. Post-epidemic monitoring should be conducted in addition to telemetry and other monitoring programs described in Section 3 to detect a novel threat. For environmental toxins, field investigations should be carried out to determine if undiscovered point sources persist on the island. The length of such investigations should be determined by the IMT. For an epidemic, a set of foxes should be tested at frequent intervals (e.g., monthly) for the underlying disease through analysis of blood samples or other diagnostic tool as determined by the IMT. Other potential reservoir species, especially island skunks should also be tested for the disease. NCI resident or migrant species which serve as potential reservoirs for rabies include skunks (Blanton et al. 2010), house mice, deer mice (Childs et al. 1997), and bats (Daoust et al. 1996, McQuiston et al. 2001). Potential reservoir species for CDV include skunks (Williams 2001) and marine mammals (Kuiken et al. 2006, Australia Department of Agriculture, Fisheries and Forestry 2000). Skunks may also serve as a potential reservoir species for CAV (Grate et al. 1987) or CPV (Bakker et al. 2006) on NCI. Post epidemic monitoring should continue for the longest known incubation period of a disease unless determined otherwise by the IMT.

8. References.

Acton AE, Munson L, Waddell WT. 2000. Survey of necropsy results in captive red wolves *Canis rufus*, 1992-1996. J. Zoo Wildl. Med. 31:2-8.

Aguilar A, Roemer G, Debenham S, Binns M, Garcelon D, Wayne R. 2004. High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. Proc. Natl. Acad. Sci. U.S.A. 101:3490-3494.

All About Hantaviruses [Internet]. [updated 2004 Aug 5]. Atlanta (GA): Center for Disease Control; [cited 2010 Dec 15]. Available from http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/prevent5.htm

Appel, MJ. 1987. Canine Distemper Virus. In: Appel MJ, editor, Virus infections of carnivores. New York: Elsevier Science. p 133-159.

Australia Department of Agriculture, Fisheries and Forestry. 2000. Importation of animals of the suborder Pinnipedia into Australian zoos. Final Import Risk Analysis Report. Canberra (Australia).

Bakker, V.J., Van Vuren, D.H., Crooks, K.R., Scott, C.A., Wilcox, J.T., Garcelon, D.K., 2006. Serologic survey of the island spotted skunk on Santa Cruz Island. Western North American Naturalist 66 (4) 456-461.

Barker IK, Parrish CR. 2001. Parvovirus infections. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Blackwell. p 131-146.

Blanton JD, Palmer D, Rupprecht CE. 2010. Rabies surveillance in the United States during 2009. J. Am. Vet. Med. Assoc. 237:646-657.

Cabasso VJ. 1962. Infectious canine hepatitis virus. Ann. N. Y. Acad. Sci. 101:498-514.

Cabasso VJ. 1981. Infectious canine hepatitis. In: Davis JW, Karstat LH, Trainer DO, editors. Infectious diseases of wild mammals. 2nd ed. Ames (IA): Iowa State University Press. p 191-195.

Carman PS, Povey RC. 1985. Pathogenesis of canine parvovirus-2 in dogs: Haematology, serology, and virus recovery. Res. Vet. Sci. 38:134-140.

Childs JE, Colby L, Krebs JW, Strine T, Feller M, Noah D, Drenzek C, Smith JS, Rupprecht CE. 1997. Surveillance and spatiotemporal associations of rabies in rodents and lagomorphs in the United States, 1985-1994. J. Wildl. Dis. 33:20-27.

Clifford DL, Mazet JAK, Dubovi EJ, Garcelon DK, Coonan TJ, Conrad PA, Munson L. 2006. Pathogen exposure in endangered island fox *Urocyon littoralis* populations: implications for conservation management. Biol. Conserv. 131:230-243.

Coonan T. 2009a. San Miguel Island fox update [abstract]. In: Island Fox Working Group 11th Annual Meeting; 2009 June 16-18; Ventura (CA): National Park Service. P.4.

Coonan T. 2009b. Santa Rosa Island fox update [abstract]. In: Island Fox Working Group 11th Annual Meeting; 2009 June 16-18; Ventura (CA): National Park Service. P.5.

Coonan T, Schwemm C, Garcelon DK. 2010. Decline and recovery of the island fox. New York: Cambridge University Press. 212 p.

Daoust, PY, Wandeler AI, Casey GA. 1996. Cluster of rabies cases of probable bat origin among red foxes in Prince Edward Island, Canada. J. Wildl. Dis. 32:403-406.

Davidson WR, Appel MJ, Doster GL, Baker OE, Brown JF. 1992. Diseases and parasites of red foxes, gray foxes, and coyotes from commercial sources selling to fox-chasing enclosures. J. Wildl. Dis. 28:581-589.

Deem SL, Spelman LH, Yates RA, Montali RJ. 2000. Canine distemper in terrestrial carnivores: a review. J. Zoo Wildl. Med. 31:441-451.

Doak D, Clifford D, Bakker V. 2006. Rabies modeling to estimate adequate collar numbers and response times. In: Spencer W, Rubin E, Stallcup J, Bakker V, Cohen B, Morrison S, Shaw R. 2006. Framework monitoring plan for the San Clemente Island fox with specific recommendations for the 2006 field season. San Francisco (CA): The Nature Conservancy. p G1-G8.

Ebert D, Bull JJ. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? Trends Microbiol. 11:15-20.

Garcelon DK, Wayne RK, Gonzales BJ. 1992. A serologic survey of the island fox *Urocyon littoralis* on the Channel Islands, California. J. Wildl. Dis. 28:223-229.

Garcia and Associates. 2009. Island Fox *Urocyon littoralis clementae* Monitoring and Research on Naval Auxiliary Landing Field, San Clemente Island, California. DRAFT Large Report Option Period 2; 2009 Field Season. Prepared for: Environmental Operations and Planning Environmental Requirements and Assessments N45. Cooperative Agreement Number N62473-07-D-3202; Task Order 0001, CLIN 0101.

Grate SJ, Bartz CR, Montali RJ. 1987. Canine Adenovirus Type 1. In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science Publishers BV. p 407-415.

Hanlon CA, Niezgoda M, Rupprecht CE. 2007. Rabies in terrestrial animals. In: Jackson AC, Wunner WH. Rabies. 2nd ed. San Diego (CA): Elsevier Inc. p 201-258.

Hudgens B, Ferrara F, Garcelon D 2007. Remote Monitoring of Island Foxes. Arcata (CA): Institute for Wildlife Studies. DoD Legacy Project #06-308.

Hudgens B, Ferrara F, Garcelon D. 2008. Digital Radio-Telemetry Monitoring of San Nicolas Island Foxes. Arcata (CA): Institute for Wildlife Studies. DoD Legacy Project #07-308.

Hudgens BR, Garcelon DK. 2010. Induced changes in island fox *Urocyon littoralis* activity do not mitigate extinction threat posed by a novel predator. Oecologia. 2010 Sep 3. [Epub ahead of print: http://dx.doi.org/10.1007/s00442-010-1761-7].

Ikeda Y, Nakamura K, Miyazawa T, Chen M-C, Kuo T-F, Lin JA, Mikami T, Kai C, Takahashi E. 2001. Seroprevalence of canine distemper virus in cats. Clin. Diagn. Lab. Immunol. 8:641-644.

Inoue T, Nonaka N, Kanai Y, Iwaki T, Kamiya M, Oku Y. 2007. The use of tetracycline in anthelmintic baits to assess baiting rate and drug efficacy against *Echinococcus multilocularis* in foxes. Vet. Parasitol. 150:88-96.

Johnson MR, Boyd DK, Pletscher DH. 1994. Serologic investigations of canine parvovirus and canine distemper in relation to wolf *Canis lupus* pup mortalities. J. Wildl. Dis. 30:270-273.

King J, Duncan C. 2009. Santa Catalina Island fox recovery program update [abstract]. In: Island Fox Working Group 11th Annual Meeting; 2009 June 16-18; Ventura (CA): National Park Service. P.3.

Krebs JW, Mandel EJ, Swerdlow DL, Rupprecht CE. 2005. Rabies surveillance in the United States during 2004. J. Am. Vet. Med. Assoc. 227:1912-1925.

Kuiken T et al. 2006. The 2000 canine distemper epidemic in Caspian seals *Phoca caspica*: pathology and analysis of contributory factors. Vet. Pathol. 43:321-338.

McCue PM, O'Farrell TP. 1988. Serological survey for selected diseases in the endangered San Joaquin kit fox *Vulpes macrotis mutica*. J. Wildl. Dis. 24:274-281.

McQuiston JH, Yager PA, Smith JS, Rupprecht CE. 2001. Epidemiologic characteristics of rabies virus variants in dogs and cats in the United States, 1999. J. Am. Vet. Med. Assoc. 218:1939-42.

Montali RJ, Bartz CR, Bush M. 1987. Parvoviruses. In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science. p 419-428.

Munson L. 2010. Diseases of island foxes. In: Coonan T, Schwemm C, Garcelon DK editors. Decline and recovery of the island fox. New York: Cambridge University Press p 129-143.

O'Keefe K, Antonovics J. 2002. Playing by different rules: the evolution of virulence in sterilizing pathogens. Am. Nat. 159:597-605.

Pollock RVH. 1982. Experimental canine parvovirus infection in dogs. Cornell Vet 72:103-119.

Rosatte RC, Power MJ, MacInnes CD, Campbell JB. 1992. Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. J. Wildl. Dis. 28:562-71.

Rupprecht CE, Stohr K, Meredith C. 2001. Rabies. In: Williams ES and Barker IK, editors. Infectious Diseases of Wild Mammals. 3rd ed. Ames (IA): Blackwell. p 3-26.

Sanchez, J. and B. Hudgens. 2012. Vaccination and monitoring strategies for San Clemente Island Foxes. Abstract and Presentation in: The Fourteenth Annual Meeting of the Island Fox Working Group, Sheraton Four Points Hotel, Ventura California. June 12-14, 2012.

Schmidt GA, Hudgens BR, Garcelon DK. 2005. Island Fox Monitoring and Research on Naval Auxiliary Landing Field, San Clemente Island, California, Final Report May 2004-June 2005. Arcata (CA): Institute for Wildlife Studies.

Shen DT, Gorham JR. 1980. Survival of pathogenic virus at 5C and 25C. Vet Med Small Anim Clin 75:69-72.

Smith GC, Wilkinson D. 2003. Modeling control of rabies outbreaks in red fox populations to evaluate culling, vaccination, and vaccination combined with fertility control. J. Wildl. Dis. 39:278–286.

Spencer W, Rubin E, Stallcup J, Bakker V, Cohen B, Morrison S, Shaw R. 2006. Framework monitoring plan for the San Clemente Island fox: with specific recommendations for the 2006 field season. San Francisco (CA): The Nature Conservancy.

Thulke HH, Eisinger D, Selhorst T, Muller T. 2008. Scenario-analysis evaluating emergency strategies after rabies re-introduction. Dev Biol (Basel) 131:265-272.

Timm SF, Munson L, Summers BA, Terio KA, Dubovi EJ, Rupprecht CE, Kapil S, Garcelon DK. 2009. A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes *Urocyon littoralis catalinae*. J. Wildl. Dis. 45:333-343.

U.S. Coast Guard 2006. Incident Management Handbook. Washington, D.C. Publication P3120.17A.

U. S. Department of Transportation 2002. Hazardous Materials: Revision to Standards for Infectious Substances. Federal Register. Vol. 67:53118-53144.

Van Riper C, Van Riper SG., Goff ML, Laird M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monogr 56:327-344.

Williams ES. 2001. Canine distemper. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Iowa State University Press. p 50-59.

Wolstenholme R. 2009. Santa Cruz Island fox recovery program update [abstract]. In: Island Fox Working Group 11th Annual Meeting; 2009 June 16-18; Ventura (CA): National Park Service. P.6.

Woods LW. 2001. Adenoviral diseases. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Iowa State University Press. p 202-212.

Woodworth BL et al. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proc. Natl. Acad. Sci. U.S.A. 102:1531-1536.

APPENDIX A: Background information on major disease threats.

Rabies: Rabies is a lyssavirus that causes acute encephalomyelitis in mammals around the world, although it is primarily found in carnivores and bats (Rupprecht et al. 2001). Lyssaviruses replicate quickly in mammalian neural tissue; however they breakdown quickly in the external environment. They are rapidly inactivated by exposure to ultraviolet radiation (i.e., direct sunlight), heat, drying, desiccation, and repeated freeze-thaw cycles. Under certain conditions this infectious virus has been recovered from carcasses exposed to the elements months after the host animal died (such as carcasses frozen in winter; Rupprecht et al. 2001). "Rabies" refers to the rabies virus itself, as well as several related species in the genus *Lyssavirus* that cause similar symptoms and are considered interchangeable in terms of diagnosis, disease prevention, and management. Each of the viruses is found most often in a particular host reservoir species, but appear capable of infecting any mammal. Terrestrial mammals are only known to be reservoirs of the rabies virus, while bats act as reservoirs for rabies and all other rabies-related viruses (World Health Organization 2005). The primary wildlife reservoirs of rabies in the United States are raccoons; skunks; bats and red, arctic, and gray foxes (Blanton et al. 2010). Cats are the leading domestic animal in the United States diagnosed with rabies (Krebs et al. 2005). This is probably not due to cats being more susceptible to the virus, but rather because the regulations requiring domestic dogs to be vaccinated do not extend to cats, as well as the tolerance most communities have of free-ranging cats compared to free-ranging dogs (Hanlon et al. 2007). In developed countries cats are usually infected with the predominant circulating wildlife variant in the area (McQuiston et al. 2001). Rabies surveillance is focused on humans and domestic species; wildlife are more difficult to monitor. The current system is basically one of passive surveillance for wildlife, where sick or dead mammal species that have had encounters with humans are tested for rabies infection by local or state public health departments. This is especially true of bats, which are primary reservoirs and often escape detection due to their high mobility (Rupprecht et al. 2001).

In all species, the rabies virus can be transmitted through contact with the brain, neural tissue, or saliva of an infected individual. It is generally passed between hosts when an infected animal bites a susceptible host, transmitting the virus through its saliva (World Health Organization 2005). Open wounds contaminated with infectious material such as saliva are considered to be an exposure to rabies and may result in infection. Other modes of transmission that have been considered include the consumption of infected brain or salivary gland tissue, during which the virus could enter the body if bone fragments penetrated oral or esophageal tissue. Contamination of mucous membranes such as the eyes and nose, or oral exposure have also been hypothesized; however evidence for these modes of transmission mostly come from laboratory studies (Hanlon et al. 2007).

After entering a new host, the virus moves to the central nervous system (CNS) where it replicates, but the animal does not exhibit any symptoms of the disease during a 1-3 month incubation period. This period has been known to vary from several days to several years; however it usually does not last longer than 6 months. The length of incubation can depend on the location of the infectious bite, with shorter incubation periods occurring after bites to the head and neck, or highly innervated areas (Rupprecht et al. 2001). Once the virus reaches the brain, the first symptoms begin (World Health Organization 2005). It is during the clinical phase of the disease that a host animal is infectious, although the virus can be shed between 3 and 10 days prior to the onset of symptoms (Rupprecht et al. 2001). Rabies is almost always fatal once

symptoms begin, with death happening within 1-5 days. Death occurs when encephalitis and myelitis (inflammation of the brain and spinal cord, respectively) become so severe that blood flow to the brain is compromised, or when symptoms cause multiorgan failure (World Health Organization 2005, Rupprecht et al. 2001).

Several weeks after infection initial symptoms such as tingling and numbness of the skin at the exposure site, restlessness, loss of appetite, vomiting or diarrhea can occur. After 1-2 days an acute neurologic period follows (also known as "furious rabies"), which can present with behavioral changes such as a general increase in activity, increased sensitivity to stimuli, agitation and aggressiveness, head tilt or head pressing, altered vocalizations, loss of caution, and altered activity cycles (e.g., nocturnal animals becoming active during the day). It is during this phase that transmission of most rabies infections occur, because the virus is present in the salivary glands and heightened aggression and abnormal behavior increase the likelihood that an infected animal will attack and bite another animal, thereby transmitting the virus. The virus can also affect the nervous system, causing facial asymmetry, choking, drooling, drooping of the lower jaw, and the pupils to be unequal in size. If the CNS is affected, the resulting symptoms could include hyperactivity, disorientation, excessive sensitivity to light, a loss of coordination, and seizures. The animal may progress to a paralytic phase ("dumb rabies") in which symptoms include lethargy, frequent urination or incontinence, decreased spinal reflexes, lack of coordination, and impaired movement. Paralysis during this phase may lead to respiratory or cardiac failure. While all of these symptoms are common, they may present in any combination, or not at all. An animal may skip the "furious" phase and progress directly to the paralytic "dumb" phase, die immediately after initial symptoms begin, or die without any outward signs of disease at all (Rupprecht et al. 2001).

There is currently no specific treatment for rabies, though at least one human treated with a combination of therapies has survived the disease. Pre-exposure vaccines are available for humans, domestic animals, and wildlife, and take the form of injectable and oral doses. Most of what is known about the efficacy of rabies vaccines comes from domestic animals. An animal is considered vaccinated 28 days after initial vaccination, when peak virus antibody titers are reached. Immunity lasts anywhere from 1-3 years depending on the vaccine used. There are currently no USDA licensed biologics for post-exposure treatments in animals.

The pre-exposure vaccine currently being used to vaccinate island foxes is Merial's Imrab 3, which is an injectable vaccine that utilizes a killed virus. In dogs it is safe for puppies at least 3 months of age. The recommended vaccination protocol for dogs is to revaccinate one year after the initial vaccination, then every three years after that. It is one of the most commonly used vaccines in zoological collections and recommended by the American Association of Zoo Veterinarians for non-domestic carnivores. The only oral vaccine for rabies currently licensed in the United States is RABORAL V-RG, manufactured by Merial, Inc. for use in raccoons and coyotes (Centers for Disease Control and Prevention 2008). It utilizes the vaccinia virus as a live virus vector, which expresses only the rabies antigen and therefore cannot cause the disease (Merial 2008, Brochier et al. 1989). The vaccine is delivered in one of two forms of bait: a "fishmeal polymer" consisting of a fishmeal outer shell incasing a vaccine filled sachete, and a "coated sachete" that has bait adhered directly to the vaccine sachete in order to reduce size and weight (Merial 2008). The fishmeal polymer bait contains the biomarker tetracycline hydrochloride, which binds to calcium in growing bone and teeth and can be used to confirm whether or not an animal has consumed a bait. This is done by removing an upper canine or premolar, cutting it longitudinally into 100–200 mm sections, mounting the sections on glass

sides, and examining them under fluorescence microscopy (Rosatte et al. 1992, Inoue et al. 2007). If tetracycline was ingested, there will be a golden-yellow line visible in the dentin or cementum. RABORAL V-RG baits should be shipped and stored refrigerated (2-7^{o}C), and are not intended or approved for use in domestic pets (Merial 2008).

Oral baits, trap-vaccinate-release (TVR), and culling have been used alone and in various combinations in countries worldwide in an effort to control or eradicate rabies. Many states consistently drop oral baits over large areas of land as part of long-term rabies control. In outbreaks where the spread of rabies is in one direction, and geographic features allow for the isolation of rabies-free areas, creating a linear vaccine barrier ahead of the wave of rabid animals can be effective. If no geographic features are present, and the spread of disease radiates outward in multiple directions, a circular barrier of vaccine can be used (Merial 2008). If an isolated case of rabies is identified, a common response is point infection control (PIC). This consists of first reducing the population of hosts immediately surrounding the infected individual (usually by culling) to remove any animals who may have been infected as well as reduce the population to a density at which virus transmission becomes unlikely. Next TVR is initiated in a circle around the population reduction area, and oral baits are dropped in an outer ring past the TVR area. This method is effective for controlling 'hot spots' of disease (Rosatte et al. 2007), and has been successfully used with RABORAL V-RG oral baits to limit the spread of an outbreak of raccoon rabies in Ontario, Canada (Rosatte et al. 2001). However, PIC may not be useful for island foxes because of the extremely small size of the islands compared to areas of the mainland where this method has been used. For example, the PIC response to raccoon rabies in Canada had a radius of 25km (~1963km^2; Rosatte et al. 2001). Additionally, the host density threshold required to maintain a rabies epidemic in mainland foxes has been estimated to be 0.63 individuals/km^2 for red foxes in Europe (David et al. 1982), 0.3 individuals/km^2 for foxes in Siberia (Tyul'ko and Kuzmin 2002), and 0.4-0.7 individuals/km^2 for red foxes in the UK (Smith and Wilkinson 2003). Culling runs the risk of disturbing territory boundaries, causing animals to move from high density to low density areas. This increased movement could lead to greater incidences of disease transmission (Smith and Wilkinson 2003).

There are several factors to consider when using oral baits to control or eliminate rabies in wildlife. First, the bait being used must be appropriate for the target species in terms of bait consumption and subsequent disease protection. Cliquet et al. (2008) fed RABORAL V-RG fishmeal polymer baits to captive red foxes, and found 100% bait consumption and 80% seroconversion. Of the animals that seroconverted, 100% survived a rabies challenge. Second, the density of baits must be high enough that a sufficient portion of the target population is reached, as well as to allow for consumption by non-target species. Oral baits successfully eradicated the arctic fox rabies variant in red foxes in Ontario, Canada after seven years when aerially dropped in lines spaced 1-2km apart, with an average of 20 baits/km^2 (MacInnes et al. 2001). Also in Canada, RABORAL V-RG baits were aerially dropped in an effort to control raccoon rabies from 1999-2006. In 69-86% of red foxes tested, evidence of bait acceptance (tetracycline biomarker) was evident at 75 baits/km^2, and 50-67% at 150 baits/km^2. The acceptance rate at 150 baits/km^2 is lower than expected, most likely because some of the baits used at this density did not contain a tetracycline biomarker, so acceptance of these baits could not be measured. Bait acceptance by raccoons ranged from 26 to 83%, and was generally higher for adults compared to juveniles, males compared to females, and at 150 baits/km^2 compared to 75 baits/km^2 (Rosatte et al. 2008). Third, the method of bait distribution should be appropriate for the species and geographic area being targeted. Dropping baits from aircraft is the best way

to evenly cover large areas; however, hand baiting can be more effective in habitat types that are difficult to penetrate, or in urban areas (Rosatte et al. 2007). Also, distributing baits in widely spaced lines can miss individuals if the target species has a small home range or limited movement (Rosatte et al. 2007). Fourth, the cost of purchasing and distributing oral baits must be considered. In the previously mentioned raccoon rabies control project in Canada, the average cost to aerially distribute RABORAL V-RG (including bait cost, air distribution, staff salary, equipment, etc.) was $147.39 USD/ km^2 at 75 baits/km^2, and $273.53 USD/ km^2 at 150 baits/km^2 (Rosatte et al. 2008). The cost of dropping oral baits in Ohio from 1997 to 2000 at 79-93 baits/km^2 ranged from $102.00 to $261.00 USD/ km^2 (Foroutan et al. 2002).

Canine Distemper Virus (CDV): CDV is a morbillivirus that occurs worldwide and can infect all members of the order Carnivora. North American hosts include coyotes, wolves, red foxes, gray foxes, San Joaquin kit foxes, black-footed ferrets, striped skunks, black bears, and raccoons (Williams 2001). There is large variation in the susceptibility of species, even within families. For example, gray foxes are extremely susceptible and rarely survive CDV (Appel 1987b), while red foxes show more resistance (Williams 2001).

Transmission generally occurs from inhalation of aerosol droplets from the respiratory tract, or contact with oral and ocular fluids of a sick animal (Deem et al. 2000, Williams 2001). Acutely infected dogs shed the virus beginning approximately 7 days post-exposure. The virus is also shed from skin, feces, and urine; however, these are considered less likely routes of transmission between individuals due to the fragility of the virus outside a host animal (Williams 2001). The virus has been shown to survive for 48 hours at 25 degrees Celsius and 14 days at 5 degrees Celsius (Shen and Gorham 1980), but is generally short-lived in the environment due to its susceptibility to ultraviolet light, heat, desiccation, and disinfectants (Deem et al. 2000).

Dogs infected with CDV can present with a disease ranging from no visible symptoms whatsoever, to a serious disease having a 50% mortality rate (Williams 2001). More severe cases are often caused by secondary infections due to protozoa or bacteria. Once inhaled, the virus moves to the lymph system where it replicates for several days. After approximately one week post-infection, the virus moves via the blood stream to the epithelial and CNS tissue (Deem et al. 2000), as well as systemic lymphatic tissues, the digestive system, and the liver (Williams 2001). Virus shedding begins once CDV reaches the blood stream and epithelial tissue, regardless of whether or not clinical symptoms develop (Appel 1987b).

The incubation period of CDV in dogs ranges from one week to over one month. The first sign of systemic infection is a fever, which may be unnoticeable (Appel 1987b). Several days later a second fever peaks, usually concurrent with a pale yellow nasal discharge, conjunctivitis, anorexia, a dry cough progressing to a moist cough, hyperkeratosis (thickening of the epidermis due to increased amounts of keratin) of the footpads and nose, depression, vomiting, and diarrhea (Appel 1987b, Williams 2001). Leukopenia (a decrease in white blood cells) is always present, and may be followed by gastrointestinal and respiratory signs (Appel 1987b). Symptoms of the CNS may happen concurrently with primary symptoms, or follow within 1-5 weeks of recovery (Williams 2001). These may include convulsions, seizures, vestibular signs (e.g., loss of balance), impaired movement or paralysis, aimless wandering, muscle twitching, head tilt, involuntary eye movements, and an increase in sensitivity to sensory stimuli (Appel 1987b, Greene and Appel 1998). CDV is usually fatal in dogs that develop symptoms of the nervous system, and if recovery occurs there are often residual symptoms (Appel 1987b). Symptoms are usually similar in non-domestic carnivores, such as raccoons and

foxes, to those found in domestic dogs, but may also include aggressiveness and lack of fear, and can therefore be confused with rabies (Helmboldt and Jungherr 1955).

The severity of the disease is determined by the host's immune response. Clinical symptoms may never develop if there is a strong antibody response. If the host's antibody response is weak, symptoms may develop but recovery usually occurs approximately 3 weeks post-infection. The virus is cleared from the body except for the lungs, skin, and CNS, and the animal may shed the virus for several months. If there is little to no antibody response, the disease becomes severe by 2 to 3 weeks post-infection, and death occurs by 3 to 4 weeks. If an animal recovers, virus shedding may occur for 2 to 3 months until the virus is completely cleared from the body. There is no cure for canine distemper, so treatment focuses on treating the symptoms of the disease. Animals that recover from CDV are considered to have a lifelong immunity to the disease (Williams 2001).

Chronic distemper encephalitis (also called "old dog encephalitis") is a disease that can be found in adult dogs with no history of systemic canine distemper. The disease is caused by an inflammatory reaction associated with chronic infection of the CNS with CDV. Symptoms include lack of coordination, compulsive movements, and progressive neurologic signs. The canine distemper antigen has been found in the brain of some dogs with this disease; however, they are not infectious (Kahn 2008).

Injectable CDV vaccines are made with a MLV or inactivated virus (Williams 2001). Vaccine-induced cases of canine distemper are rare, but do occur. One such case resulted in the death of four European mink (*Mustela lutreola*) at a zoo in Russia (Sutherland-Smith et al.1997). The animals were vaccinated with an avian-origin vaccine, developed distemper symptoms within 16-20 days, and all had died by 26 days post-vaccination. Another case resulted in the death of an island fox held in captivity at the Hogle Zoo in Utah (Coonan et al. 2010). Most vaccine-induced cases of canine distemper occur after the use of vaccines made from canine-tissue-adapted strains of CDV. MLV vaccines from egg-adapted and primate tissue strains have been safer when used in non-domestic species (Deem et al. 2000). Island foxes are currently vaccinated with Merial's Purevax Ferret Distemper Virus vaccine, which utilizes recombinant canarypox vector. In ferrets it is recommended for use in animals at least 8 weeks old, with primary vaccination consisting of 3 injections at 3-week intervals, then revaccination once annually thereafter. There are no oral baits available for CDV.

Canine Adenovirus Type 1 (CAV-1): CAV-1 is found worldwide, and causes Infectious Canine Hepatitis (also known as Fox Encephalitis, Rubarth's Disease, or Hepatitis Contagiosa Canis; Grate et al. 1987). It can infect members of Canidae, Mustelidae, and Ursidae (Woods 2001), with natural infections having been reported in red and gray fox, coyote, wolf, black bear, polar bear (*Ursus maritimus*), raccoons, and striped skunks (Grate et al. 1987). Antibodies to CAV have been found in foxes on all of the Channel Islands except for Santa Catalina (Clifford et al. 2006, Garcelon et al. 1992). Of 78 serum samples collected from San Clemente foxes between 2001 and 2003, 58 (74.4%) of them tested positive for CAV antibodies, with mature foxes being 7 times more likely than young foxes to have been exposed (Clifford et al. 2006). The high prevalence of CAV in island foxes from islands where domestic dogs have been removed, and the continued naivety of Santa Catalina foxes to the virus (where domestic dogs are still present), suggest that CAV is enzootic or there may be another source of the virus (Garcelon et al. 1992). Canine Adenovirus Type 2 (CAV-2) is the only other canine adenovirus recognized, but has not been reported to cause disease outside domestic dogs (Grate et. al. 1987).

CAV-1 is not airborne (Appel 1987a); rather it is shed by infectious animals in urine, feces, and secretions of the eyes and nose (Woods 2001). Virus shedding in the urine can continue for at least 6 months (sometimes over a year) after recovery from the disease (Appel 1987a). The primary modes of transmission are direct contact between hosts and ingestion of material contaminated with the virus (Grate et al. 1987). The virus itself is fairly stable in the environment, retaining infectiousness for 10-13 weeks at room temperature (Cabasso 1962). The virus remains infectious in tissue culture for 26-29 days at 37 degrees Celsius, but is inactivated within minutes at temperatures above 50 degrees Celsius (Ikegami et al. 1959), by ultraviolet light, or common disinfectants (Appel 1987a).

Studies in dogs show that CAV-1 infection most often occurs through the tissue in the back of the throat. The virus moves into the bloodstream, and from there infects the internal organs and CNS (Woods 2001). In foxes, the incubation period of CAV-1 is 2-6 days, after which initial symptoms such as anorexia and nasal discharge begin. Symptoms progress to diarrhea, hyperexcitability, seizures, paralysis, and coma (Cabasso 1981). Due to the similarity of symptoms, animals presenting with neurological signs can appear to be suffering from rabies or CDV. Death may occur after a short clinical course or occur suddenly with no symptoms (Woods 2001). Non-domestic carnivores in general may have titers without presenting with clinical signs, or carry the virus with minimal signs (Grate et al. 1987). Most of the data on CAV-1 related mortality in non-domestic carnivores comes from foxes in fur farms. These animals show extreme susceptibility at less than 6 months of age, with mortality as high as 80% (twice that of adults; Greene et al. 1930). Few non-domestic carnivores besides foxes have been known to survive CAV-1 (Grate et al. 1987). Foxes that survive the disease once demonstrate a high degree of immunity if inoculated a second time (Greene et al. 1930). Dogs that survive CAV-1 infection are considered to be immune for the rest of their lives (Appel 1987a).

Injectable vaccines for CAV-1 and CAV-2 are available in killed and MLV forms. Gore et al. (2005) showed that a multivalent, modified live CDV, CAV-2, and Canine Parvovirus (CPV) vaccine (Continuum DAPP, Intervet) provided domestic dogs with 100% protection from infection from CAV-1 for up to 3 years. Twenty-three seronegative pups were vaccinated at 7 and 11 weeks. Dams were also seronegative; therefore there was no risk of maternal antibodies interfering with vaccine efficacy. Following 3 years of isolation, vaccinated and unvaccinated control dogs were exposed to CAV-1, CPV, and CDV. None of the vaccinated animals developed clinical signs of CAV, while 5 out of the 6 animals in the control group developed severe clinical signs, and 3 died. Subsequent challenge of vaccinated animals with CPV and CDV did not result in any clinical signs of disease. It should be noted that this study also demonstrates that the CAV-2 vaccine is effective in preventing CAV-1 infection. This makes CAV-2 vaccines a potentially safer option for wildlife since they are not susceptible to the CAV-2 virus itself (therefore they cannot develop vaccine-induced disease). Modified live vaccines have also been used successfully in foxes, and provide solid and long lasting immunity (Greene et al. 1930, Greene et al. 1935).

Canine Parvovirus Type 2 (CPV): CPV is found worldwide and known generally as “canine parvovirus” or CPV. This virus can infect domestic dogs and wild canids, including all wild members of the genus *Canis*. In North America CPV has been confirmed in coyotes, red and grey wolves, island skunk, and foxes (red, gray, island, and kit; McCue and O’Farrell 1988, Davidson et al. 1992, Garcelon et al. 1992, Johnson et al. 1994, Acton et al. 2000, Bakker et al. 2006).

CPV is shed in feces, and in dogs the greatest amount of virus is shed between 4 and 10 days after infection (Carman and Povey 1985). Dogs no longer transmit the virus approximately 4 weeks after recovery (Pollock 1982). The virus is very stable in the environment, and can remain infectious in feces at room temperature for 6 months (Pollock 1982). Transmission is generally by a fecal-oral route, probably from exposure to marking sites and latrines rather than direct contact between animals (Barker and Parrish 2001).

Clinical signs in wildlife are similar to those in dogs, and appear approximately 4 to 5 days post-exposure. After entering the body the virus replicates in the back of the throat, tonsils, and other lymph tissues. The virus moves through the body in the bloodstream and lymph cells (Barker and Parrish 2001). The disease is always accompanied early on by a severe decrease in the number of white blood cells in the bloodstream (Montali et al. 1987). All parvoviruses affecting carnivores cause intestinal lesions, the severity of which is determined by age, co-infections, and overall health. These lesions lead to diarrhea (usually containing blood and mucus), vomiting, dehydration, and fever, the severity of which depends on the amount of damage to the intestine (Barker and Parrish 2001). Other symptoms include anorexia, lethargy, vomiting, or there can be sudden death with no symptoms (Montali et al. 1987). Antibody production is detectable around the time the first symptoms begin. If the intestinal damage is not too great, animals can recover completely. Animals that die usually do so within four to five days of the onset of symptoms. CPV generally attacks the developing heart tissue of infected dogs between 3 and 8 weeks, and can cause death from inflammation of heart muscle (Barker and Parrish 2001).

Vaccines containing a killed virus are not consistently effective across Canidae, usually require isolation and repeat vaccinations to work (Montali et al. 1987), and are also not consistently commercially available. MLV vaccines are used in dogs and similar wild canids such as coyotes (Greene et al. 1984) and African wild dogs (*Lycaon pictus*; Spencer and Burroughs 1990). An MLV vaccine resulted in good protection against CPV in captive litters of maned wolves (*Chrysocyon brachyurus*) and bush dogs (*Speothos venaticus)* without isolation from the rest of the colony (Montali et al. 1987).

References

Acton AE, Munson L, Waddell WT. 2000. Survey of necropsy results in captive red wolves (*Canis rufus*), 1992-1996. J. Zoo Wildl. Med. 31:2-8.

Appel MJ. 1987a. Canine Adenovirus Type 1 (infectious canine hepatitis virus). In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science. p 29-43.

Appel, MJ. 1987b. Canine distemper virus. In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science. p 133-159.

Barker IK, Parrish CR. 2001. Parvovirus infections. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Blackwell. p 131-146.

Blanton JD, Palmer D, Rupprecht CE. 2010. Rabies surveillance in the United States during 2009. J. Am. Vet. Med. Assoc. 237:646-657.

Brochier B et al. 1989. Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. J. Wildl. Dis. 25:540-547.

Cabasso VJ. 1962. Infectious canine hepatitis virus. Ann. N. Y. Acad. Sci. 101:498-514.

Cabasso VJ. 1981. Infectious canine hepatitis. In: Davis JW, Karstat LH, Trainer DO, editors Infectious diseases of wild mammals. 2nd ed. Ames (IA): Iowa State University Press. p 191-195.

Carman PS, Povey RC. 1985. Pathogenesis of canine parvovirus-2 in dogs: Haematology, serology, and virus recovery. Res. Vet. Sci. 38:134-140.

Centers for Disease Control and Prevention. 2008. Compendium of animal rabies prevention and control, 2008. Morbidity and Mortality Weekly Report. 57(No.RR-2):1-9.

Clifford DL, Mazet JAK, Dubovi EJ, Garcelon DK, Coonan TJ, Conrad PA, Munson L. 2006. Pathogen exposure in endangered island fox (*Urocyon littoralis*) populations: implications for conservation management. Biol. Conserv. 131:230-243.

Cliquet F, Barrat J, Guiot AL, Caël N, Boutrand S, Maki J, Schumacher CL. 2008. Efficacy and bait acceptance of vaccinia vectored rabies glycoprotein vaccine in captive foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes rocyonoides*) and dogs (*Canis familiaris*). Vaccine. 26:4627-4638.

Coonan T, Schwemm C, Garcelon DK. 2010. Decline and recovery of the island fox. New York: Cambridge University Press. 212 p.

David JM, Andral L, Artois M. 1982. Computer simulation model of the epi-enzootic disease of vulpine rabies. Ecol Modell 15:107-125

Davidson WR, Appel MJ, Doster GL, Baker OE, Brown JF. 1992. Diseases and parasites of red foxes, gray foxes, and coyotes from commercial sources selling to fox-chasing enclosures. J. Wildl. Dis. 28:581-589

Deem SL, Spelman LH, Yates RA, Montali RJ. 2000. Canine distemper in terrestrial carnivores: a review. J. Zoo Wildl. Med. 31:441-451.

Foroutan P, Meltzer MI, Smith KA. 2002. Cost of distributing oral raccoon-variant rabies vaccine in Ohio: 1997-2000. J. Am. Vet. Med. Assoc. 220:27-32.

Garcelon DK, Wayne RK, Gonzales BJ. 1992. A serologic survey of the island fox (*Urocyon littoralis*) on the Channel Islands, California. J. Wildl. Dis. 28:223-229.

Gore TC, Lakshmanan N, Duncan KL, Coyne MJ, Lum MA, Sterner FJ. 2005. Vet. Ther. 6:5-14.

Grate, SJ, Bartz CR, Montali RJ. 1987. Canine Adenovirus Type 1. In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science Publishers BV. p 407-415.

Greene RG, Ziegler NR, Greene BB, Dewey ET. 1930. Epizootic fox encephalitis: I. General description. Am J Hyg 12:109-129.

Greene RG, Ziegler NR, Greene BB, Schillinger JE, Dewey ET, Carlson WE. 1935. Epizootic fox encephalitis: VIII. Nature of the immunity. Am J Hyg 21:366-388.

Greene JF, Bruss ML, Evermann JF, Bergstrom PK. 1984. Serologic response of captive coyotes (*Canis latrans say*) to canine parvovirus and accompanying profiles of canine coronavirus titers. J. Wildl. Dis. 20:6-11.

Greene CE, Appel MJ. 1998. Canine distemper. In: Greene CE, editor. Infectious diseases of the dog and cat. 2nd ed. Philadelphia: WB Saunders. p 9-22.

Hanlon CA, Niezgoda M, Rupprecht CE. 2007. Rabies in terrestrial animals. In: Jackson AC, Wunner WH. Rabies. 2nd ed. San Diego (CA): Elsevier Inc. p 201-258.

Helmboldt CF, Jungherr EL. 1955. Distemper complex in wild carnivores simulating rabies. Am. J. Vet. Res. 16:463-469.

Ikegami T, Konishi S, Ochi Y. 1959. Studies on infectious hepatitis of dogs. III. Properties of the virus in dog kidney tissue culture. J. Vet. Sci. 21:33-38.

Inoue T, Nonaka N, Kanai Y, Iwaki T, Kamiya M, Oku Y. 2007. The use of tetracycline in anthelmintic baits to assess baiting rate and drug efficacy against *Echinococcus multilocularis* in foxes. Vet. Parasitol. 150:88-96.

Johnson MR, Boyd DK, Pletscher DH. 1994. Serologic investigations of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortalities. J. Wildl. Dis. 30:270-273.

Kahn CM, editor. 2008. The Merck veterinary manual. 9th ed. Whitehouse Station (NJ): Merck & Co., Inc.

Krebs JW, Mandel EJ, Swerdlow DL, Rupprecht CE. 2005. Rabies surveillance in the United States during 2004. J. Am. Vet. Med. Assoc. 227:1912-1925.

MacInnes CD et al. 2001. Elimination of rabies from red foxes in eastern Ontario. J. Wildl. Dis. 37:119-132.

McCue PM, O'Farrell TP. 1988. Serological survey for selected diseases in the endangered San Joaquin kit fox (*Vulpes macrotis mutica).* J. Wildl. Dis. 24:274-281.

Merial. 2008. Control of raccoon rabies using RABORAL V-RG from Merial. Duluth (GA): Merial Limited.

Montali RJ, Bartz CR, Bush M. 1987. Parvoviruses. In: Appel MJ, editor. Virus infections of carnivores. New York: Elsevier Science Publishers BV. p 419-428.

Pollock RVH. 1982. Experimental canine parvovirus infection in dogs. Cornell Vet 72:103-119.

Rosatte R et al. 2008. Prevalence of tetracycline and rabies virus antibody in raccoons, skunks, and foxes following aerial distribution of V-Rg baits to control raccoon rabies in Ontario, Canada. J. Wildl. Dis. 44:946-964.

Rosatte R, Donovan D, Allan M, Howes L, Silver A, Bennett K, MacInnes C, Davies C, Wandeler A, Radford B. 2001. Emergency response to raccoon rabies introduction into Ontario. J. Wildl. Dis. 37:265–279.

Rosatte RC, Power MJ, MacInnes CD, Campbell JB. 1992. Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. J. Wildl. Dis. 28:562-71.

Rosatte RC, Tinline RR, Johnston DH. 2007. Rabies control in wild carnivores. In: Jackson AC, Wunner WH. Rabies. 2nd ed. San Diego (CA): Elsevier Inc. p 595-634.

Rupprecht CE, Stohr K, Meredith C. 2001. Rabies. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Iowa State University Press. p 3-26.

Shen DT, Gorham JR. 1980. Survival of pathogenic virus at 5C and 25C. Vet Med Small Anim Clin. 75:69-72.

Smith GC, Wilkinson D. 2003. Modeling control of rabies outbreaks in red fox populations to evaluate culling, vaccination, and vaccination combined with fertility control. J. Wildl. Dis. 39:278-286.

Spencer JA, Burroughs REJ. 1990. Antibody response in wild dogs to canine parvovirus vaccine. S. Afr. J. Wildl. Res. 20:14-15.

Sutherland-Smith MR, Rideout BA, Mikolon AB, Appel MJG, Morris PJ, Shima AL, Janssen DJ. 1997. Vaccine-induced canine distemper in European mink, *Mustela lutreola*. J. Zoo Wildl. Med.. 28:312-318.

Tyul'ko ZS, Kuzmin IV. 2002. Simulation of rabies epizootic process in fox populations at a limited carrying capacity of biotopes. Russian Journal of Ecology. 33:331-337.

Williams ES. 2001. Canine distemper. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Iowa State University Press. p 50-59.

Woods LW. 2001. Adenoviral diseases. In: Williams ES, Barker IK, editors. Infectious diseases of wild mammals. 3rd ed. Ames (IA): Iowa State University Press. p 202-212.

World Health Organization. 2005. WHO Expert Consultation on Rabies: First Report. Geneva, Switzerland: WHO Press.

APPENDIX B: Determining Viral Shedding from Tissue Swabs.

Protocols are in place to determine viral shedding for both CDV and CPV from tissue swabs which can be taken in the field. In addition to standard fox trapping and handling equipment, collecting tissue swabs requires polyester swabs and an RNA preservative. The products RNA*later* and ethanol are recommended for CDV swabs and available through a number of research chemical outlets. High grate ethanol is recommended for CPV fecal swabs. Once an animal is in hand, the collection protocol is as follows:

1) Swab conjunctiva of each eye with single swab and place into tube with RNA*later*[*].
2) Swab nose (be sure to get sample of any discharge if present) -- place into same tube with the ocular swab.
3) If oral exam possible - take soft palate swab. With bite bar in place - swab as far back as safely possible on the upper roof of the mouth. Try to get past the hard palate to the soft tissues; close to causing a little gag reflex. Goal of this swab is to capture any sinus drainage coming down. Place swab into a tube with RNA*later**.
4) Fecal swab - since this is for parvovirus - we put this swab into high grade ethanol.

[*] If RNA*later* not available - all swabs can be put in high grade ethanol

Samples will remain intact for up to 3 days at 37° C, 7 days at 25° C a month if refrigerated at 4° C. Samples to be archived should be stored at -20° C or colder.

The labs recommended by the Island Fox Conservation Group to process tissue swabs are:
CDV: Cara Wademan at the UC Davis RT-PCR lab:
http://www.vetmed.ucdavis.edu/vme/taqmanservice/contact.html
PH:503-792-7991

CPV: Mourad Gabriel at the Integrated Ecology Research Center:
PH:707-845-7847
http://www.iercecology.org/contact/

APPENDIX C: Trapping, transport and quarantine protocols to prevent disease spread.

Trapping protocols. Capturing and transporting foxes during an epidemic must be done with great care to avoid unintentionally spreading an infectious disease. The only infectious disease among those considered here known to be transmitted to humans is rabies. Because symptoms may be similar for rabies and other diseases, and because of the extreme human health risk posed by the disease, all personnel involved in fox trapping, transportation and care should be vaccinated against rabies. Some pathogens, particularly canine distemper virus, canine adenovirus, and canine parvovirus (the most environmentally persistent) are shed into the environment and may spread through re-use of traps and trapping materials. Traps should never be placed near a carcass, feces, or other discharge. Traps should be removed from the field after they have been occupied by a fox or skunk, apparently triggered by an animal trying to get to bait (e.g., signs of digging around a closed but empty trap, a trap knocked out of place), or signs of fresh fox activity within 1 meter of the trap (feces, tracks etc.). Traps should be cleaned thoroughly with bleach and trap accessories (e.g., bait dishes, bite-bars, burlap covers) should be sterilized or replaced after each time they capture or are visited by a fox before they are re-used, placed near other traps to be used, placed near quarantine facilities or placed where fox trapping or fox care personnel may come into contact with them.

As with traps, other equipment and materials used during the capture or transport of foxes or skunk during a possible epidemic should be sterilized after each use. It is also important that personnel involved in trapping and animal care do not transmit potential pathogens on their clothing or persons. There are several precautions that should be enforced to avoid pathogen transmission. First, outer layers, including gloves and apron, should be changed after each fox handled and either disposed of or cleaned thoroughly before reuse (box D1). Second, different clothing, especially footwear, should be worn in the field, in and around quarantine facilities and during every-day activities. Finally, hands should be thoroughly washed after each fox handled, after all trips to the field, and before and after entering the quarantine site. Hand sanitizer is a very effective substitute if water/soap are not available for washing.

Once an animal has been captured it should be evaluated in the field for potential signs of infection. If rabies is suspected, or if a captured animal seems unusually aggressive as the trap is approached, the animal should be anesthetized before it is handled. Once removed from the trap, the animal should be visually inspected for disease symptoms. Finally the animal should be examined by a qualified veterinarian prior to being incorporated into the quarantined population. If examinations cannot be conducted at the time of trapping, foxes should be held in isolation until they can be examined. Animals in the quarantined population should be kept isolated from each other as well as from animals outside the quarantine facility; at a minimum all animals should be initially held in isolated "receiving" pens for a long enough period to determine that they are not infected (1 week – 3 months based on incubation periods described in Appendix A) prior to interact with other quarantined animals. In the ideal case where quarantined animals are maintained in isolation, these initial receiving pens may be the same as the long-term holding

Box D1: Proper Care of Outerwear. Compiled from interviews with expert working group.

- The outer (contaminated) surface of a gown should only be touched with gloves.
- After unfastening ties, peel the gown from the shoulders and arms by pulling on the chest surface with gloves.
- Remove the gown, avoiding contact between the outer surface and clean surfaces.
- Ball the gown up for disposal while keeping the contaminated surface on the inside.
- Remove gloves and wash hands.
- If body fluids have soaked through the gown, promptly remove the contaminated clothing and wash the skin.
- Store and transport used outerwear in sealed plastic bags while in the field or quarantine site.
- Gloves should always be worn when handling soiled laundry.
- Bedding and other laundry should be machine washed with standard laundry detergent and machine dried.
- Separate storage and transport bins should be used for clean and dirty laundry.

pens within the quarantine facility. Infected animals should be euthanized as directed by a qualified veterinarian.

Transport protocols. Apparently healthy captured foxes should be transported to a receiving facility in individual containers (e.g., the box-trap they were captured in) with no more than one animal transported per vehicle. If multiple animals have been trapped from an area, they should be kept at least 10 feet apart in the field until they can be safely transported to initial receiving pens. While captured animals are being held in waiting for safe transport, they should be closely

monitored to 1) ensure they do not display any symptoms of infection and 2) ensure they do not come into contact with potentially infected free-ranging animals. Transport cages and vehicles should be cleaned with bleach or other disinfecting agent after each use. The same precautions described above to avoid transmitting pathogens on persons or clothing while trapping apply to transporting animals.

Quarantine methods to prevent spread infectious disease. Quarantined foxes need to be housed in a way that prevents exposure both from free-roaming infected animals and from asymptomatic but infected animals within the quarantine facility. Preventing exposure from free-roaming infected animals is primarily accomplished by enclosing the quarantine site with a fox-proof and skunk-proof fence. It is also important that fox-care personnel do not transport pathogens on their clothing or persons. Preventative measures include: maintaining separate teams for fox care and for field activities (i.e., trapping, transport, monitoring), requiring fox-care personnel to wear sterilized clothing and footwear when entering the quarantine facility, and requiring fox-care personnel to wash hands before and after each visit to the quarantine facility.

Preventing exposure from undetected infected animals within the quarantine facility is primarily accomplished by: immediately removing animals that become symptomatic from the facility, keeping animals separated far enough apart that aerosolized particles cannot travel from one housing to another, and sterilizing food and water bowls and other materials used in fox care before each use. Biological materials that are suspected of potentially infectious, including feces, carcasses or bodily discharge from known ill animals, should put in biohazard bags and are disposed of by companies licensed to take those materials. Storage on and removal from NCI should follow the same procedures as used by NCI medical facilities for biohazard materials.

Again, it is important that fox-care personnel do not transport infections on their clothing or persons. Preventative measures include: requiring personnel to don sterilized outerwear (e.g. aprons, caps, gloves) each time a fox is approached, and to remove outerwear before entering areas where sterilized materials are stored, requiring personnel to wash hands, faces and exposed skin after handling each fox or cleaning holding pens and before approaching another fox.

Quarantine facility required materials.

Structures:

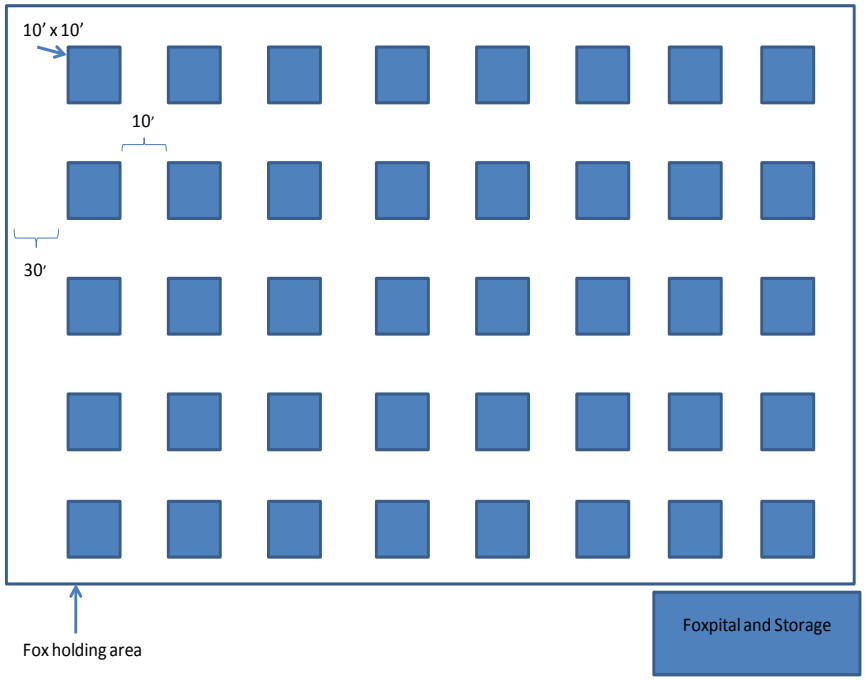
Perimeter fencing (1" mesh 6' ×1000')
Anti-climbing top and bottom (1000' × 36" aluminum flashing bottom, 28" aluminum flashing top)
Electric fencing wire (1000')
Solar electric fence charger or electric fence charger + battery
Ready-made pens (100)
Pen tops

Pen supplies (for 100 pens):

den box/crate
2 sets stainless steel food and water dishes
pass through food and water drawer
boot scrubber
Enhancement

Facility supplies:

Food dispenser and scoop
Pen cleaning supplies (pooper scooper, dispenser etc.)
Dirty laundry storage/transport container
Clean laundry/fresh disposable cover containers
Gloves, aprons, rubber boots, boot covers
Veterinary supplies
Fox body bags
Biological specimen A and B containers and labels



APPENDIX D: Shipping procedures

Shipping biological specimens through commercial or government (U.S. Postal Service) carriers requires special packaging and labels. General guidelines are provided on a number of university and medical lab web pages (Table A1). Below is a brief summary of the requirements for Category A (potentially infectious disease, e.g., rabies) and Category B (noninfectious to humans) .

Table A1. Online resources describing requirements for shipping biological specimens.

source	URL
North Carolina State University	http://www.ncsu.edu/ehs/dot/Bio_shipping.pdf
Harvard University	http://research4.dfci.harvard.edu/ehs/Biosafety/2002-ship.pdf
University of New Hampshire	http://www.unh.edu/ehs/pdf/UNH-Shipping-Biological-Materials.pdf
University of California	http://www.universityofcalifornia.edu/compaudit/researchcomp/exportctrls/documents/shpmnt_biomat_man.doc
Mayo Clinic	http://www.mayomedicallaboratories.com/specimen-transport/intltest/infectious.html http://www.mayomedicallaboratories.com/specimen-transport/intltest/noninfectious.html

Infectious Disease suspected: Biological Specimen Category A

Category A infectious substances are capable of causing permanent disability, life threatening or fatal disease to humans or animals when exposure to them occurs. Category A infectious substances have two shipping names: “Infectious substances, affecting humans” (UN 2814) or “Infectious substances, affecting animals” (UN 2900). Specimens may be shipped to necropsy lab via Fed Ex or USPS. Labels may be obtained from a number of commercial vendors.

Packaging

Category A infectious substances must be tripled packaged and compliant with IATA Packing Instruction 602 detailed in Figure C1. The maximum quantity of Category A infectious substance that can be shipped by air in one package is 4 L or 4 kg. The maximum allowable quantity on passenger aircraft is 50 ml or 50 g.

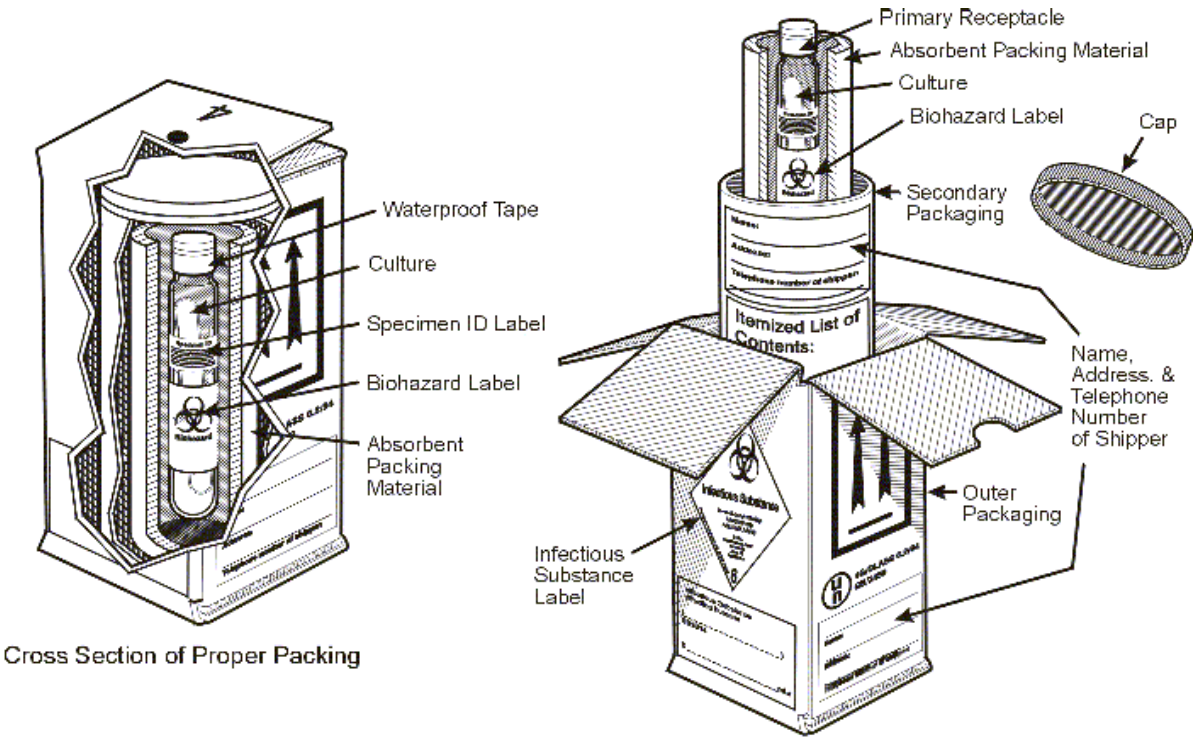
Labeling

The outer container of all Category A infectious substance packages must display the following on two opposite sides:

- Sender’s name and address
- Recipient’s name and address
- Infectious substance label
- Proper shipping name, UN number, and net quantity of infectious substance
- Name and telephone number of person responsible for shipment

• Cargo Aircraft Only label when shipping over 50 ml or 50 g
• Class 9 label, including UN 1845, and net weight if packaged with dry ice

Figure C1. Packing and labeling of infectious substances (Biological Specimen Category A).



1. Packages must bear UN specification mark
2. Shipments must be prepared so they arrive in good condition and pose no hazard to humans or animals during transport
3. Triple packaging consisting of watertight primary receptacles, watertight secondary packaging and an outer packaging of sufficient strength to meet the design test types (9 meter drop test, puncture test)
4. Primary receptacle or secondary packaging capable of withstanding a 95Kpa internal pressure differential
5. Absorbent material sufficient to absorb the entire contents of the shipment
6. An itemized list of contents must be included between the secondary and outer packaging
7. Name and number of the person responsible for the shipment must appear on the package
8. Minimum dimension 100mm

No evidence of infectious disease: Biological Specimen Category B

Category B infectious substances are infectious but do not meet the criteria for Category A. Category B infectious substances have the proper shipping name “Biological Substance, Category B” and the identification number UN 3373. Specimens may be shipped to necropsy lab via Fed Ex, UPS or USPS. Labels may be obtained from a number of commercial vendors.

Packaging

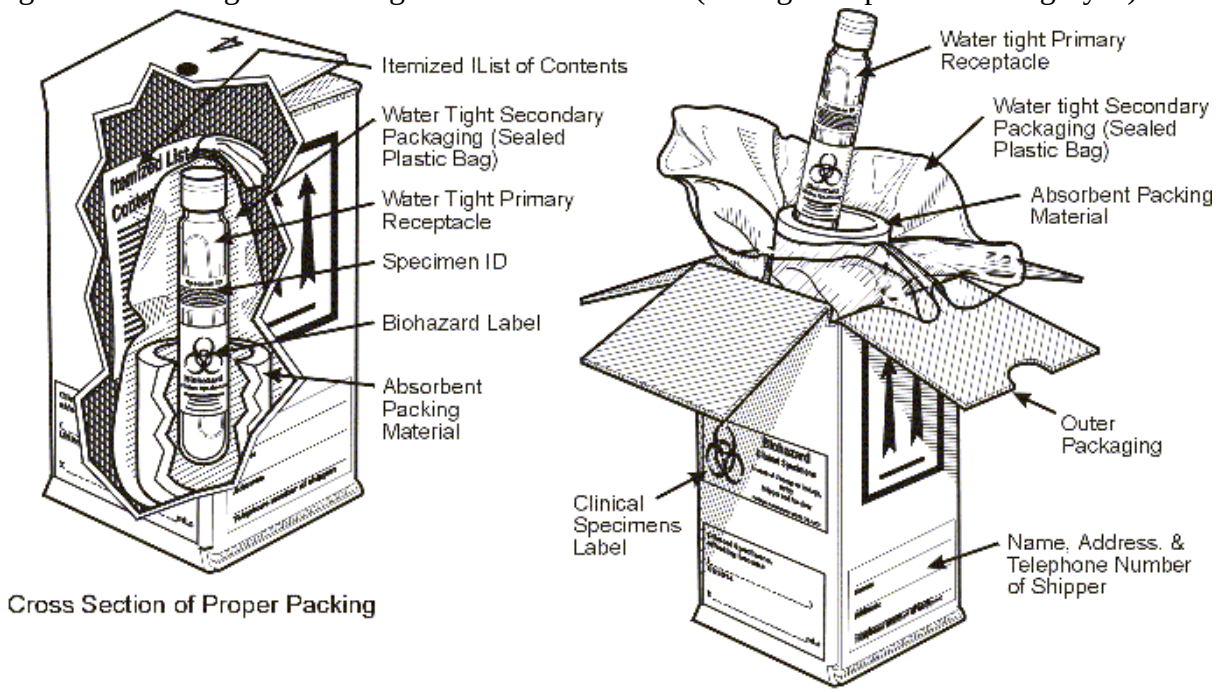
Category B infectious substances must be tripled packaged and compliant with IATA Packing Instruction 650 detailed in Figure C2. The maximum quantity for a primary receptacle is 500 ml or 500g and outer packaging must not contain more than 4 L or 4 kg.

Labeling

The outer container of all Category B infectious substance packages must display the following on two opposite sides:

- Sender’s name and address
- Recipient’s name and address
- The words “Biological Substance, Category B”
- UN 3373 label
- Class 9 label, including UN 1845, and net weight if packaged with dry ice

Figure C2. Packing and labeling of clinical substances (Biological Specimen Category B).



1. Packages must be of good quality, strong enough to withstand the rigors of transport
2. Triple packaging consisting of leak proof primary receptacles (for liquid shipments), silt proof primary receptacles (for solid shipments), leak proof secondary packaging, outer packaging of sufficient strength to meet the design type test (1.2 meter drop test)
3. For liquid shipments, primary receptacle or secondary packaging capable of withstanding a 95Kpa internal pressure differential
4. Absorbent material sufficient to absorb the entire contents of the shipment
5. An itemized list of contents must be included between the secondary and outer packaging
6. “Biological Substance, Category B” must appear on the package
7. Minimum dimension 100mm