ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5th Edition

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Workbook Organized by:
Omaha's Henry Doorly Zoo
Conservation Breeding Specialist Group/SSC/IUCN
Veterinary Specialist Group/SSC/IUCN

In collaboration with:
New Zealand Department of Conservation
Auckland Zoo
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This workbook was developed in part due to the visionary and generous direct contributions of the following institutions:

American Association of Zoo Veterinarians
Audubon Park and Zoological Garden
Lincoln Park Zoological Gardens
Omaha's Henry Doorly Zoo
San Diego Zoo
White Oak Conservation Center and the Gilman Foundation
Wildlife Conservation Society

The Conservation Breeding Specialist Group wishes to extend their appreciation to these institutions for their support.

A special thanks to **Ventana Systems** for permitting access to and use of their Vensim software for this project and to **Palisade Corporation** for permitting the use of their @Risk Software.

A contribution of the IUCN/SSC Conservation Breeding Specialist Group.

Armstrong, D., R. Jakob-Hoff, and U. S. Seal (editors). 2003. Conservation Breeding Specialist Group (SSC/IUCN). 2001 *MOVIMIENTOS DE ANIMALES Y RIESGO DE ENFERMEDAD:* ***LIBRO DE TRABAJO. 2003*** CBSG, Apple Valley, MN.

Additional copies of *MOVIMIENTOS DE ANIMALES Y RIESGO DE ENFERMEDAD:* ***LIBRO DE TRABAJO. 2003*** can be ordered through the IUCN/SSC Conservation Breeding Specialist Group, 12101 Johnny Cake Ridge Road, Apple Valley, MN 55124. email:office@cbsg.org. web:www.cbsg.org

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Acknowledgement

This workbook has been developed through the effort of a significant number of supporters and people active in conservation activities throughout the world. The people donated their time, their resources and most importantly their thoughts and ideas to this project. The Conservation Breeding Specialist Group (CBSG) initiated a series of workshops in 1991 at the National Zoo in Washington, DC intended to reach this product. The workshop series progressed through a large one in Oakland in 1992 prior to the American Association of Zoo Veterinarians meeting there and more recently through smaller workshops at Pretoria and at Cincinnati in 1999. In 2000 a concerted effort was made to synthesize the concepts and ideas that had been accumulated in the previous workshops into a working tool for broad distribution to conservation programs around the world. This final series of three intensive workshops began at Omaha in March of 2000 and then went to New Orleans in September of 2000. The series concluded with a workshop at White Oak Plantation in June of 2001. These workshops and the completion of this workbook is attributable to both the participants who worked diligently throughout the workshops, their employers who gave them the opportunity to participate and to the institutions that provided direct financial support for these final three workshops. The supporting institutions include the American Association of Zoo Veterinarians, Audubon Park and Zoological Garden, the Henry Doorly Zoo, the Lincoln Park Zoological Gardens, the San Diego Zoo, White Oak Conservation Center and the Wildlife Conservation Society. The tireless participants are listed below. Many of these people worked in more than one of the workshops to produce this product. We would like to extend our sincere thanks to the sponsors and the participants of the final three workshops, as well as the many others who contributed their expertise and support through the entire history of this project.

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A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

1. INTRODUCTION

Introduction

The workbook and the tools contained within are designed to provide a framework for a wide range of individuals with many different kinds of experience and expertise to think about and address the issue of disease and how it relates to wildlife populations and animal translocation projects. These tools are not designed to provide statistically valid, mathematically defensible answers to scientific questions. They are designed to enable professionals involved in day to day decision making about wildlife management to better equip themselves to make reasonable decisions to benefit wildlife and its conservation. The tools go through several concurrent evolutions as they develop. They begin on a very fundamental level with regard to disease and risk analysis and they progress in complexity and mathematical rigor. They also begin on a very basic level with regard to the technology required to use them, initially pencil and paper, and progress to more complex software programs that require the use of a computer and some significant training to apply. Most importantly perhaps, the tools begin on a very intuitive level of assessment and progress through a series of transitions to a more qualitative assessment and finally to very quantitative methods of assessment. The tools are designed to be flexible and modifiable according to each situation, to enable professionals to incorporate not only published, statistically valid data but also to be able to make reasonable decisions when there is no data available and to capture valuable information available from field or clinical experience.

Conceptual Development

The health of endangered species, both in the wild and in captivity, could be seriously impacted by common and emerging pathogens. Animal health experts, conservation biologists, regulatory and trade officials, and natural resource agencies are all faced with implementing risk management strategies in the face of relatively little existing information. Risk analysis is a growing field concentrated on accumulating and organizing existing information in order to prioritize relative risks to support decision-making in the face of uncertainty.

Disease is increasingly recognized as a significant risk factor in conservation programs involving animal movements such as reintroduction or translocation. Disease risk poses threats not only to the species on which programs are focused but also to other species that share the habitat. The concern over disease processes and their impact extends across diverse areas of interest including the fields of conservation biology, wild and zoo conservation management and veterinary medicine as well as to agricultural medicine and human medical fields. However disease risk has proven to be complex and difficult to assess and quantify in the context of a conservation program. The growing recognition that disease issues can profoundly effect the viability of populations and consequently the success or failure of conservation programs has led to diverse efforts by individuals and groups to develop some rational means to 1) assess the risks that disease poses to these programs, 2) develop well reasoned understandings of the factors and issues involved and 3) make reasonable decisions based on these assessments.

The philosophy of zero risk has posed an unattainable goal for needed animal movement actions in wildlife conservation programs. The need for a comprehensive, unified, and broadly applicable set of tools was agreed by all of the participants in both year 2000 workshops in their stated individual goals for the workshop and was more completely described during the workshops in terms of disease biology, data analysis and decision making tools, and communicating risk analysis information for action.

RISK CONCERNS FOR MOVEMENT OF ANIMALS

March 31, 2000
Omaha, Nebraska

PREAMBLE

Risk concerns in moving animals for conservation or wildlife management includes three groups of primary disease issues. This analysis is based upon the recognition that a zero risk tolerance philosophy does not meet the needs for decision making in conservation programs. However, there is not a comprehensive agreed, unified, broadly applicable set of tools such as protocols, models, policies or guidelines to assist assessment of disease risk associated with needed animal movement decisions.

I. DISEASE BIOLOGY

1. Multiple biological issues in addition to disease complicate the analysis of risks associated with moving animals.
Issues may include:
a) Impact on social structure of resident population and ecosystem,
b) Habitat carrying capacity, interspecies completion's?? competition
c) Other factors that need to be identified for the individual situation.

2. The impact of diseases on our ability to implement conservation actions is poorly understood.
Issues may include:
a) Impact of disease on successful outcome of animal movement is not appreciated.
b) Effects of diseases on population dynamics and population viability, on natural life history of the focus species, and on the species community,
c) Human health impacts.
d) Need to focus on populations rather than the individual animal.
e) Management actions during movements may increase the concentration of pathogens in the population being moved.
f) Public perceptions and politics.
g) Treatment may induce problems or disease or be ineffective.

3. Information on the epidemiology (e.g. dynamics of intra- and interspecific transfer) and pathogenesis (e.g. susceptibility, development of immunity) of diseases is frequently limited.
Issues may include:

a) Captive "closed" settings to "open" natural habitats
b) Infectious vs. non-infectious disease (need to consider the source and destination populations)
c) Clinical vs. pathologic aspects
d) Prevalence and incidence
e) Impact of land management practices that may promote disease transmission
f) Morbidity, mortality, case fatality

4. Information on the status of disease in source and destination populations is limited.
Issues may include:

a) What diseases exist that can affect an animal?
b) Which of those might be introduced in a translocation or reintroduction?
c) What are the consequences of moving those diseases to other species being discussed, e.g., what might spread to domestic species and other wildlife?
d) What reverse risk diseases are present in the area that might affect the species in question?

5. Diagnostic tests often are not, or can not be, used prior to transfer of animals, which may pose a serious threat to the viability of populations. Without test results there will be a failure to treat, vaccinate, or reject the moved animals.
Issues may include:

a) Appropriate diagnostic tests and standards for many different diseases and species are not available.
b) Consistent surveillance/temporal sampling for diseases over time is not done.
c) Knowledge needed for correct interpretation of diagnostic tests is not available: e.g., detection may be evidence of infection but does not indicate presence of disease. Vaccination may complicate interpretation.
d) Knowledge of sensitivity and specificity of test not available, making interpretation difficult.
e) Experimental controls or adequate sample sizes to obtain needed data are not available.
f) Non-invasive testing methods are not available.
g) Access to quality laboratories to conduct diagnostic testing is not available.

II. DATA ANALYSIS / DECISION MAKING TOOLS

1. Integrated tools to assist risk identification, characterization, assessment and management decision- making are not readily available.
Selection of analysis and decision assistance tools needs to consider:

a) International availability and accessibility.
b) Flexible, dynamic and layperson friendly format.
c) Use existing tools where possible (i.e., do not "reinvent the wheel" with existing tools).
d) Incorporation and assessment of cost: benefit of doing translocation, reintroduction, release, repatriation, restocking, transfer and rehabilitation in the context of global wildlife conservation and biodiversity.
e) Development of acceptable risks within the context of limited resources.
f) Incorporation of knowledge to set priorities (i.e., in order to address threats so that we know how much energy to put into disease assessment).
g) Is the analysis directed at a particular species, disease or ecosystem?

2. Appropriate data to apply to the selected tools are often limited or are not easily accessible.
Selection of data to include in analysis tools needs to consider:

a) Use of consistent terminology.
b) Access to available data for the international community.
c) The establishment of centralized databases.

3. Target users and interpreters of the tools and the information produced need to be a part of the process of development of the tools.
Selection of the target users needs to consider including:

a) Multidisciplinary professional team including biologists, veterinarians, behaviorists, geneticists, toxicologists, epidemiologists, infectious disease specialists.
b) Multidisciplinary animal management team (including veterinarians, wildlife managers,
c) Policy makers (politicians, sociologist, public, special interest groups, activists).

III. COMMUNICATING RISK ANALYSIS INFORMATION FOR ACTION

Introduction
Wildlife and zoo managers and policymakers need essential and valid information to make informed decisions. Communicating the conservation value of information to decision makers in an understandable and compelling way is essential for this process of using risk assessment information to be a part of the decision making process. Some of the problems to solve for achieving a successful communication strategy were identified.

1. **It is difficult to get managers and decision makers to agree on an acceptable level of risk.**

   What information and communication process needs to be considered, e.g., what role do diseases play in wild populations?

2. **Access to applicable data and the results of analyses, especially information from international sources and those on the Internet is difficult.**

3. **Communication between risk assessment specialists (modelers) and those with data on disease in populations is needed.**

4. **Disease risk data are not considered by decision-makers and stakeholders because the data are not communicated in a user-friendly way to facilitate action.**

5. **Effective methods and mechanisms for dissemination and communication of information to stakeholders on disease characteristics and associated risks are not used.**

6. **People are unaware of existing tools (data, models, protocols, etc.) and how they could be used to identify and minimize disease risks.**

   It is difficult even with this knowledge and understanding to get people to use the tools.

7. **Science-based policy and management decision-making are hampered in geopolitically-pressured environments.**

8. **There needs to be general agreement that acceptable risk results in higher probability of negative movement events that a zero risk strategy.**

Scope and Magnitude of Disease in Species Conservation

Linda Munson, Dept Veterinary Pathology, Microbiology and Immunology, University of California, Davis, CA 95616

Most diseases impacting animal conservation are infectious in origin, although genetic and toxic diseases also influence population viability. Infectious diseases have caused significant losses across all taxa, but several notable catastrophic epidemics have occurred in endangered wild carnivore populations.

The canine distemper virus epidemic in the Serengeti ecosystem resulted in the loss of approximately one third of the principal large predators (lions and hyenas) and uncounted numbers of other carnivores. The rapid spread and extensive impact of this virus in species that previously were not affected by CDV was the culmination of altered viral virulence and ecological factors, such as the high density of lions, their congregation in prides, and interactions with hyenas at kill sites. Canine distemper also was responsible for near extinction of the black footed ferret in the US and recently has extirpated the Channel Island fox population on the eastern half of Santa Catalina Island.

Several African wild dog populations throughout Africa have been extirpated by epidemics of rabies and CDV. The northern Serengeti population in the Masai Mara disappeared in 1989 because of a rabies epidemic, and southern Serengeti populations disappeared in the early 1990s from a disease epidemic of unknown etiology. Populations in Botswana and South Africa also have been recently extirpated by rabies and CDV. Although the wild dog may appear uniquely predisposed to fatalities from infectious disease, it is more likely that their complex social interactions facilitates viral transmission and enhances traumatic fatalities from aggression. Regardless, infectious diseases clearly impede efforts to conserve small populations of carnivores in close proximity to human habitation where the persistence of rabies and CDV in domestic dog populations constitutes a recurrent threat.

Canine distemper also has affected endangered carnivores in captivity, and the scarcity of safe, efficacious CDV vaccines has hampered preventive medicine programs. In captivity these populations are at even greater risk because infectious agents are more concentrated and genetic diversity is often more restricted than are wild populations. Captive stress confounds these other risks by modulating the immune response to infectious agents.

The scope and magnitude of infectious disease epidemics in carnivores are greatly influenced by behavior and ecology. Large carnivore populations are small and fragmented because of habitat restrictions imposed by human conflicts. This habitat fragmentation limits emigration and immigration, leading to progressively reduced genetic diversity, which has the potential to increase susceptibility to disease. Breeding behavior in many large carnivores (e.g. alpha animals) further restricts genetic diversity. Because large carnivores are at the top of food chain, they can receive concentrated doses of infectious agents through their prey, for example lions acquiring *Mycobacteria* from Cape buffalo in South Africa or cheetahs acquiring anthrax from infected meat. Also, the sequestering of their young in dens results in exposures to high concentrations of pathogens at a susceptible age. Infectious agents can also be concentrated and transmitted at sites of territory marking. Furthermore, conspecific social behavior and

interspecific competition with other predators at kill sites facilitates infectious disease transmission. Taken together, these features enhance the impact of disease on carnivore populations and need to be considered when assessing the risk of disease.

The prototype species in which diseases have hampered conservation efforts is the cheetah. Results of more than ten years of pathology surveillance of both wild and captive populations have disclosed high prevalences of unusual degenerative diseases (leukoencephalopathy, veno-occlusive disease and glomerulosclerosis) of unknown etiology, as well as unusually severe or persistent forms of infectious diseases (e.g. *Helicobacter* gastritis, feline herpes virus, feline corona virus, canine parvovirus, anthrax). They also appear more predisposed to cryptococcosis and notoedric mange than other felids. Their response to many common infectious agents is characterized by a florid, inappropriate immune response leading to immune-mediated disease or a failure to clear intracellular agents leading to persistent infection. These responses are features of an immune response modulated by cortisol (Th1 to Th2 shift), a hypothesis supported by evidence that captive cheetahs have four-fold higher levels of cortisol than wild cheetahs. Their reduced genetic diversity also may be a contributing factor to their predisposition to develop diseases.

Because of this propensity to develop diseases, conservation efforts for cheetahs have been hampered. Deaths of genetically valuable animals have prevented implementation of the SSP Master Plan. Concerns that moving cheetahs among zoos will expose them to new biotypes of pathogens, as well as increase stress has also impeded captive conservation efforts. The concerns have validity because deaths from veno-occlusive disease and feline infectious peritonitis have followed movement of cheetahs between facilities, suggesting some link between these events. In wild populations, translocations increase exposure to anthrax or notoedric mange as well as potentially causing stress-induced immunodysfunction. Also, translocated cheetahs can introduce infectious agents, such as feline leukemia virus or feline coronavirus, to cheetahs in the recipient environment. These risks, however real or hypothetical, are better managed then ignored and should not hold hostage efforts to conserve these populations.

Assessing the risk of disease in animal conservation requires knowledge of species responses to important pathogens, as well as an understanding of disease pathogenesis and predisposing factors. No population can be free of disease, but minimizing diseases that cause catastrophic losses, impede reproduction or target genetic founders is a reasonable goal. Also, diseases that are an outcome of conservation strategies (e.g. injury or hyperthermia from translocation or exposure to toxic or infectious agents during translocation) can be reduced if identified.

An important aspect of assessing the disease risk to a species is distinguishing whether a species actually has a disease or simply harbors or has been exposed to potentially infectious agents. Many “disease surveys” have been simply surveys of antibody titers, which have no direct link to morbidity or mortality effects. Pathology, as a translational science, continues to be the most reliable means to determine if disease is an outcome of exposure. As new technologies enhance our abilities to detect toxins and infectious agents, this linkage of agent with disease condition will become more critical. Otherwise all programs will be paralyzed by information without context. Because experimental reproduction of disease is not possible in most wild animals, the “Koch’s postulate of pathologists”, which is identifying the pathogen (or toxin)

within an appropriate lesion, becomes most useful. Applying these criteria should minimize inappropriate concern for organisms that are minimally pathogenic or part of a species normal flora.

Transmission or exacerbation of disease through conservation strategies is a valid concern, but these concerns should not thwart conservation programs. Reasonable policies can be designed through predictive models that can be applicable to situations with limited hard data. Such risk assessment models could be invaluable for identifying high-risk actions, thereby providing a more objective method for making informed decisions about animal translocations. Alongside the development and application of these tools, however, should be an equal commitment to acquire as much new information as possible on diseases in the populations of concern

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I want to begin by noting some of the disease and translocation issues of particular importance to Australasia.

Disease Issues

- The long geographic isolation of the continent combined with very stringent border controls have ensured that several diseases of major importance elsewhere have not yet gained a foothold in Australasia. These include such major causes of mortality and/or debility as Foot and Mouth Disease, Rabies, Avian Influenza and Newcastle Disease Virus (although there has been a recent outbreak of NDV in poultry in Eastern Australia)

- Within NZ and, to a lesser extent Australia, there are large numbers of off-shore islands which are being strategically used to isolate threatened native animal populations from endangering factors such as introduced predators.

- Many wildlife populations in both countries are fragmented even within the main land mass resulting in ecologically isolated meta-populations.

- While many diseases are known to be harbored by wildlife there is very little documented data on the impact of these diseases on the wildlife populations themselves (there are a few exceptions such as Chlamydiosis in koalas whose impact on fertility and mortality has been extensively studied)

- Mass die-offs due to infectious diseases are not a feature in this region (discounting those that are part of natural ecological cycles such as the annual die-off of all adult male dunnarts, *Sminthopsis stuartii*).

- Possibly as a consequence of the lack of overt visibility of wildlife mortalities there is currently no focused effort to determine wildlife health profiles (i.e. disease distribution and prevalence) for specific species, populations or ecological communities.

Translocation Issues

- Translocation of threatened species as part of species recovery strategies are common and increasing, particularly wild to wild and captive to wild translocations. However, recognition of associated disease risks is only emerging at this time and pre-shipment quarantine and health screening is not standard practice and funds for this are not generally budgeted.

- There are a small but growing number of veterinarians with wildlife expertise but virtually none are employed by wildlife agencies.

- Vets are also not routinely included in species recovery groups.

- In general, wildlife managers see disease threats as very minor compared to many of the more obvious endangering forces such as the impact of introduced predators and competitors, habitat loss, pollution, fires etc. Their experience with these more overt problems make them reluctant to divert their limited resources to disease detection and surveillance – a Catch 22 given that if you don't look you won't find. At Auckland Zoo, where we look a lot, we are finding infectious agents not previously described in some threatened native species (e.g. avian malaria and avian pox in NZ dotterels, *Charadrius obscurus* and avian babesiosis in North Island brown kiwi, *Apteryx australis mantelli*). In surveillance of free-living populations we are finding organisms previously only described in captive animals (e.g. coccidia in NI brown kiwi). These findings are raising the awareness of disease issues within both zoo and DOC communities and barriers to proactive action are beginning to fade.

Predicting Populations at Risk for Mortality Events

Suzanne Kennedy-Stoskopf, D.V.M. , Ph.D.

Mass mortality events capture the attention of the media and public. In aquatic ecosystems, whether it is marine mammals washed up on beaches or fish floating in rivers, people want an immediate, straightforward explanation of cause. In a rush to provide simple answers to what are usually complex questions, multi-factorial events are often overlooked or worse still ignored. The role of *Pfiesteria* in menhaden mortalities during the 1990's in North Carolina estuarine rivers and Chesapeake Bay tributaries remains controversial. This toxic dinoflagellate, dubbed the "cell from hell" by the media, does not cause the characteristic deep ulcerations around the anal pore and tail stalk, raising the issue of whether *Pfiesteria* contributes to the mortality events or merely serves as a bio-marker for other conditions which cause muscle necrosis and death. The ulcers appear to form from the inside out as deep muscle necrosis, which can be seen in the absence of ulcerations. Certain fungi have been described previously as associated with these ulcers and inoculation of *Aphanomyces invadens* can reproduce the lesions. Recently, *Kudoa*, a myxosporean parasite that causes post-mortem acceleration of muscle degeneration referred to as "soft flesh," has been implicated. None of these suggested etiologies explain the predominant localization of lesions distally. One possible hypothesis to explain this observation is that fish experience transient anoxic events and shunt blood to perfuse vital organs. Reperfusion of the ischemic muscle tissue causes oxidative stresses that trigger inflammatory reactions and subsequent deep muscle necrosis. Clearly, there is not a simple explanation to account for the recent fish mortalities in mid-Atlantic estuarine rivers.

Before mass mortality events can be placed in context, it is helpful to know the health status of the impacted population. The unusually high prevalence of ulcerative skin lesions in fishes from Chesapeake Bay tributaries and two menhaden kills in the Pocomoke River during the summer and fall of 1997 prompted the U. S. Geological Survey's National Fish Health Laboratory (Kearneysville, WV) and the Maryland Department of Natural Resources (Stevensville, MD) to conduct a broad-base study of fish health. One facet of this study assessed immune function in white perch (*Morone americana*) using a traditional functional assay and comparing it to a molecular technique to determine if newer technology could be adapted to evaluate immunosuppression within a population. A PCR-based technology has been developed to measure expression of the cytokine, transforming growth factor-beta (TGF-ß), in a wide variety of teleost fish. Enhanced expression of TGF-ß correlates with a variety of suppressed immune functions in higher vertebrate species. Immunomodulation studies demonstrated that TGF-ß expression correlates inversely with macrophage bactericidal activity in fish. Results from fish collected in the Chesapeake Bay tributaries showed the same inverse correlation. Enhanced TGF-ß expression and decreased macrophage killing occurred in certain rivers in August and October compared to June. Although there were no fish mortalities, menhaden with characteristic ulcerative myositis were observed in August and October. Identification of underlying, predisposing factors remain to be elucidated but the study is currently ongoing. The importance of the findings to date is verification of new technology to evaluate immune function within a population that is potentially more user-friendly in field applications. This technology can be adapted to other immunoregulatory proteins to provide much needed tools to assess whether populations are at risk for mortality events before they occur.

ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5th Edition

Costa Rica

28-30 April 2003

2. DISEASE LIST AND PROJECT DIAGRAM

Disease List and Project Diagram

Make a list of diseases that may be significant in your project, or all diseases you are aware of that effect any species involved in the project, or the species being translocated. Ask yourself these questions about each disease.First Exercise

1. **Fill out a project description using the form provided or creating your own format. This will be the basis on which you will build in the use of the remaining tools in this workbook.** (Translocation and Release form at the end of this section)

2. **Make a pencil and paper diagram of the steps of the project. Keep the diagram as simple as possible for now and include key steps**.

Example

In order to give you an idea of the objective, a simple hypothetical release program for reintroducing Siberian tigers from zoos back into the wild is illustrated below.

Captive tigers- multiple zoos in North America and Europe	→	Individual crates by truck to multiple airports	→	Individual crates by multiple airplanes to Vladivostock	→	Central training facility in Khabarovsk , individual caging
						↓
		Individual animal soft release enclosure at each site	←	Truck in individual crate to multiple release sites, 1 animal per site	←	One year training program, moving between small individual enclosures and large training enclosure

3. **Make a list of diseases that occur in the species being moved or in any related wild or domestic species in the release area.**
   Rank the diseases according to what you feel is their relative importance, list the reasons you think that. Record the basis for your ranking, Why do you think it should be ranked where it is. If field data, note that, if from an article note that, if the basis is from your impression, note that, record hard data when you have it.
   (If ranking becomes difficult see the Paired Ranking exercise on the next page)

   Review the following questions with regarding each disease listed.
   Do you intuitively feel that this disease is important for the animals to be moved or for animals where they are to be moved to?
   Which diseases do you need to think about more?
   Which do you need to get more information about?
   Maybe you need to do nothing more.

Paired Ranking

This is a means of producing a ranked list when it proves difficult to sort listed items into a priority list. It may be useful for an individual or a working group if the disease list is difficult to prioritize. The mechanism for carrying this technique out is very simple. As an example we will work with a limited list of three cat diseases for demonstration purposes.

1. First List the diseases in any order.

Canine Distemper
Toxascaris
Tuberculosis

2. Then define the criteria by which you will compare the diseases such as effect on the individual, potential effect on the wild population, how transmissible the disease is, etc.

3. Then compare the first disease on the list to the second and decide which is more important for the criteria you have defined and place an X to the right of that disease that you feel is most important.

Canine Distemper X
Tuberculosis
Toxascaris

4. Then compare the first disease on the list to the third and decide which is more important according to your criteria and place an x beside it.

Canine Distemper X X
Tuberculosis
Toxascaris

5. Then compare the second disease on the list to the third and repeat the exercise, placing an X by the disease you consider most important according to your criteria.

Canine Distemper X X
Tuberculosis
Toxascaris X

6. Repeat this process until all diseases on the list have been compared to all the other diseases one at a time. Then add up the number of X's by each disease and rewrite your list so that the disease with the most X's is at the top of the list.

Canine Distemper	X X	2
Toxascaris	X	1
Tuberculosis		0

This exercise can be carried out individually or can be done in a working group or can be done individually by all the individuals

Translocation and Release Project Planning and Management

Project Name:_____

Brief project narrative description:_____

_____

_____

_____

Species to be translocated:_____

_____

_____

Purpose of Project:_____

_____

_____

Origin of Animals:

Wild_____ Rehabilitation_____ Captive_____

How animals will be obtained:

How many sources of animals are there?_____

_____

_____

Free Ranging Captures?
Method Of Capture:_____

_____

_____

Rehabilitation Center?
How many of this species and related species are housed there?_____

_____

_____

Captive - Zoo or Similar:_____

How many of this species and related species are house there?_____

_____

_____

Transportation:

List all locations the animals will be in from the site of origin to the actual release:

_____

_____

_____

_____

_____

Will the animals be housed temporarily at any sites between the site of origin and the release site

_____

_____

_____

What specific means (truck, airplane, boat) will be used to move the animals in each part of the transportation?_____

_____

_____

How will the animals be housed or confined during transportation and holding at temporary locations or release sites?

_____

_____

Enclosure type:_____

Singly or in a group?_____

Will animals be exposed to other species, related or not, during any part of transportation or temporary housing?_____

_____

Which species?_____

How will the animal be fed and watered during transportation and temporary housing?

_____

_____

How will these items be obtained?_____

_____

Release Site:

Will the release be slow (soft) release or quick release:

_____

Will animals be held at the release site for acclimation or other reasons? If so how long?

_____

Is a quarantine or disease testing program planned?

Attach a description if available._____

What diseases may be present in this species or related species in the release area?

_____

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3. HAZARD IDENTIFICATION

Hazard Identification

Two different tools are available in this section. Different people may be more comfortable with one or the other. You can choose which you use, you can use both, modify whats here or you can create your own. Whichever technique you use, **it is crucial that you always record the basis for your conclusion, document your information. Transparency is key to the process.**

Introduction

The hazard identification process provides a means for limiting the number of diseases considered potentially threatening to the survivability of the animal population of concern. The process should yield information required to answer the question: are the potential effects of this disease harmful enough to warrant further assessment using other tools available. To begin the hazard identification process, one must first describe:

- the population of concern;
- the adverse outcome(s) of concern;
- the environment (econiche?) within which the population lives.
-

The above description provides a framework in which the importance of the potential hazard can more adequately be judged.

Defining hazard criteria

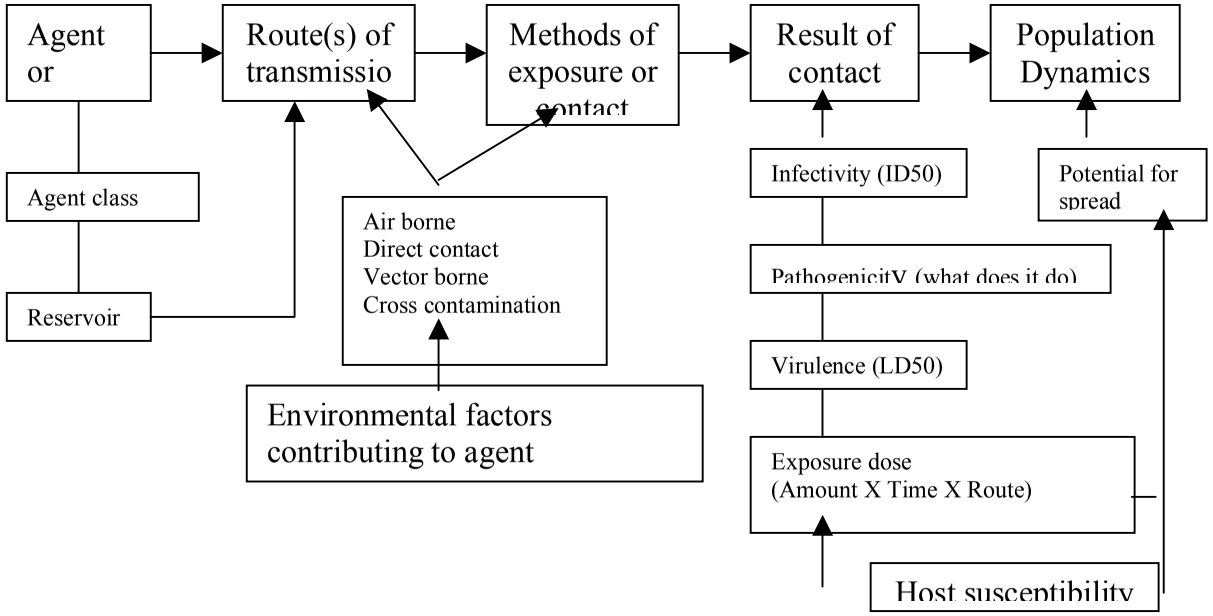
Establishing the criteria defining a hazard is the first step in the identification process. For a potential hazard to be considered a true hazard, usually all criteria must be met. The following criteria establish that a particular disease is important enough for its effects to be modeled:

- The disease reservoir exists within the habitat of the population of concern;
- Disease transmission routes for the spread of disease are likely to occur in the environmental setting outlined above;
- Exposure is likely to occur and at a level high enough to result in infection;
- Consequences of exposure include the adverse outcomes of concern considered above (e.g. high morbidity, mortality or economic impact);
- The disease is likely to spread throughout the population once it has been introduced.

Disease/Host Interaction

Hazard identification also considers characteristics of the host, the disease agent and the environment in which the disease/host interaction takes place. This interaction is depicted in Figure 1 below.

Figure 1: Important factors in disease transmission to consider in the hazard identification process



Collecting information to identify disease hazards

The table format below provides a worksheet for collecting relevant information about potential hazards. These data, including references, should be gathered for each potential hazard under consideration.

Table 1: Worksheet to more quantifiably characterize potential hazards

Agent/disease	Category (B,V,F,P)	Reservoir(s)	Agent durability in env.	Route(s) of trans.	Route(s) of potential exposure	Infectivity (ID50)	Pathogenicity	Virulence (LD50)	Host susceptibility	Potential for spread within population once introduced

Key and definitions:

Agent/disease: Name the condition, disease or specific microbiological agent

Category: Indicate the category that this disease agent falls under. Bacteria (B); Viral (V); Fungal (F); Parasitic (P).

Reservoir: Name the reservoir(s) for this disease. The reservoir is: any animate (humans, animals, insects, etc.) or inanimate object (plant, soil, feces, etc.) or any combination of these serving as a habitat of a pathogen that reproduces itself in such a way as to be transmitted to a susceptible host.

Agent durability in environment:

Describe the hardiness of the agent in the environment. Is it resistant to UV light, desiccation etc.? What are the environmental factors present in this scenario that may increase/decrease survivability of the agent?

Route(s) of transmission:

Action/event/process whereby a pathogen is passed from one individual to another. How is the agent transmitted? Horizontal vs. Vertical; Direct vs. Indirect; +/- biological vectors; +/- mechanical vectors; airborne; sexual; other routes of direct contact etc.

Potential route(s) of exposure:

Potential routes of exposure are those mentioned above in routes of transmission that are likely given the scenario outlined, as stated in the intoduction

Infectivity: The characteristic of a microorganism that allows it to infect and subsequently survive and multiply within a susceptible host. It is commonly measured using the ID50 - the median infective dose - or, the dose that will infect 50% of an exposed group.

Pathogenicity: The host-specific ability of an agent to cause disease, given infectivity, or otherwise induce pathological change in a susceptible host. The types of pathological change should be outlined as well.

Virulence: Given disease, the host-specific ability of an infectious agent to multiply in the host while inducing lesions and disease; the severity of signs given disease; the number of infected that actually come down with clinical disease; often measured by the LD50 - the median lethal dose - or, the dose that will kill 50% of the tested group.

Susceptibility: The state of being readily affected by a pathogen; a lack of resistance due to insufficient immunity because of: age, genetics, acquired conditions or any other factor making an individual more likely to become infected upon exposure. Can be in terms of individual or the group (herd immunity). Any known susceptibility factors should be outlined here.

Potential for spread:

Potential for spread depends on numerous factors including housing density, contact between animals (contact structure), herd immune status, characteristics of the agent (infectivity). This is often measured by Ro - the number of individuals that one infected individual may infect.

** definitions derived from the Dictionary of Veterinary Epidemiology. Toma B, Vaillancourt J, Dufour B, Eliot M, Moutou F, Marsh W, Benet J, Sanaa M, Michel P. Iowa State University Press, Ames. 1999.

Rough Assessment Worksheet

The intent of this worksheet is only to quickly begin the assessment process and to enable biologists and clinicians to begin to quantify and substantiate their intuitive information. This list will provide an initial basis to start a risk assessment and will begin to rank the diseases so that the most significant can be addressed as higher priorities. The list formulated will be utilized in completing further aspects of the assessment process. It is likely that the disease list will be added to as work progresses, as there is additional investigation and further input from additional sources and as new information is revealed. In addition, the relative ranking and significance of diseases will change as more specific and accurate data is applied to areas that rely only on your intuitive impression at this point.

At this stage the only concern we wish to address is to look at this in the context of how likely is it and what could be the effect of introducing a disease into a wild population through the animals to be released. We are not necessarily directly concerned about the effect on the released animals at this point. We also will focus at this point only on the species, released and wild populations, to be released at this point. It may be valuable to repeat this process thinking in terms of other species in the area that may be effected by the released species.

List all known infectious diseases you are aware of for the species being released or moved. For each disease estimate the following items based on your personal knowledge and experience. Rate each item for each disease on a scale of 1 to 5 with 1 being mild in effect or less likely to occur and 5 being serious in effect or very likely to occur. Explain your rationale for each disease element listed in the attached sheet.

Headings can be changed, make them work for your project and your group

Disease	Likelihood of Susceptibility	Likelihood of Exposure	Likelihood of Becoming Infected	Likelihood of Transmitting it to Others	Severity for the Individual if clinical	Severity for the Population	Estimated Significance to the Program

You should recognize that this chart will reflect your own personal biases and experiences. Each person that completes this sheet for any particular species may get substantially different results. Increasing the amount of input, either from other individuals or from publications, will increase the value of this initial screening.

Categories and Explanation

Likelihood of Susceptibility- What is the likelihood that an individual animal to be released will be susceptible to this disease? If it is likely that the animal to be released is able to get the disease easily if it is exposed, then you would rank it as 3. If the animal is very unlikely to get the disease even if exposed then you would rank it as 1. For instance some diseases such as tuberculosis seem to require repeated and prolonged exposure in order to infect an animal. Susceptibility is also effected by common management practices within populations or sources of animals, for example it may a known common policy in some areas to vaccinate species for some diseases, therefore making them less susceptible to diseases when exposed.

Likelihood of Exposure- What is the likelihood that the animal to be released will be or have been exposed to this disease? In general this is intended to reflect exposure prior to release so that the animal may carry the disease into the wild population. Exposure may occur at the site of origin of the animal, during transport to the release site, during holding and preparation for release. Consider elements such as what you know about the occurence of the disease in other captive animals that the release animal may be exposed to, the prevalence in wildlife or feral domestic animals the release animal may be exposed to, how well the organism survives in the environment.

Likelihood of Becoming Infected- If an animal becomes exposed, what is the likelihood that the animal will actually become infected and capable of transmitting the disease. This will include animals that are vaccinated and unlikely to become clinically ill themselves but may act as carriers in the some diseases. For instance, with the disease rabies in carnivores, if is a common management practice for animals that might enter the release program to be vaccinated already, it is very unlikely that these animals could transmit the disease even if they were exposed.

Likelihood of Transmitting it to Others- Is the disease causing organism likely to be transmitted to other individual. This will be a function of many possible factors. The social behavior of the species may be significant in that primarily solitary species such as most big cats may be less likely to transmit diseases that require direct contact to each other than species that live in social groups such as many primates or hoofstock species. Another factor may be the organisms ability to survive in the environment. Many parasites survive well and remain potentially infective for a long period of time but many viruses do not.

Severity for the Individual- If an individual in the wild population does become clinically ill with the disease, how severe is it. Does it make the animal severely ill quickly and have a high probability of killing the animal or is it a disease that rarely kills an animal or takes a long time to have a significant effect.

Severity for the Population- If a disease is likely to spread quickly through a population and kill many animals in the population then it would be considered severe for the population as well as for individuals such as rabies in wild dogs or canine distemper in lions. However some diseases may not have a significant effect on individual animals but will profoundly effect the population such as the potential effect of brucellosis in wild cattle species which would not effect an individual animal in any significant way but could profoundly effect the wild population overall by reducing reproduction significantly.

Estimated Significance to the Program- Sum the numerical values assigned to each category for this particular disease. This value will give some sense of what the importance is of this particular disease for this release program and enable you to rank the diseases with regard to your intuitive sense of their importance. Diseases which have the highest values in this column will likely be the most significant diseases to address in sorting through the steps needed in a release program.

Disease_____

Likelihood of
Susceptibility_____

_____

Likelihood of
Exposure_____

_____

Likelihood of
Becoming Infected_____

_____

Likelihood of
Transmitting it to Others_____

_____

Severity for the Individual_____

Severity for the Population_____

Estimated Significance to the Program_____

References Used_____

Rough Assessment Worksheet Example

Tiger example

This example considers the hypothetical reintroduction of captive born Siberian tigers from North American zoos into Far Eastern Russia. At this stage no quarantine or testing program is defined. The cats will come from a variety of North American zoos. The area the tigers will be released into does contain free ranging tigers as well as occasional leopards in overlapping ranges.

Disease	Likelihood of Susceptibility	Likelihood of Exposure	Likelihood of Becoming Infected	Likelihood of Transmitting it to Others	Severity for the Individual if clinical	Severity for the Population	Estimated Significance to the Program
Canine Distemper	3	1	2	1	5	5	17
Toxascaris cati	5	3	3	2	1	1	15
Tuberculosis (M. bovis)	2	1	1	1	5	1	11
Rabies	1	1	5	2	5	1	15
Feline leukemia	1	1	1	1	3	1	8
Panleukopenia	1	1	3	1	4	2	12
Feline Rhinotracheitis	1	2	5	1	3	1	13

Categories and Explanation

Likelihood of Susceptibility- What is the likelihood that an individual animal to be released will be susceptible to this disease? If it is likely that the animal to be released is able to get the disease easily if it is exposed, then you would rank it as 3. If the animal is very unlikely to get the disease even if exposed then you would rank it as 1. For instance some diseases such as tuberculosis seem to require repeated and prolonged exposure in order to infect an animal. Susceptibility is also effected by common management practices within populations or sources of animals, for example it may a known common policy in some areas to vaccinate species for some diseases, therefore making them less susceptible to diseases when exposed.

Likelihood of Exposure- What is the likelihood that the animal to be released will be or have been exposed to this disease? In general this is intended to reflect exposure prior to release so that the animal may carry the disease into the wild population. Exposure may occur at the site of origin of the animal, during transport to the release site, during holding and preparation for release. Consider elements such as what you know about the occurence of the disease in other captive animals that the release animal may be exposed to, the prevalence in wildlife or feral domestic animals the release animal may be exposed to, how well the organism survives in the environment.

Likelihood of Becoming Infected- If an animal becomes exposed, what is the likelihood that the animal will actually become infected and capable of transmitting the disease. This will include animals that are vaccinated and unlikely to become clinically ill themselves but may act as carriers in the some diseases. For instance, with the disease rabies in carnivores, if is a common management practice for animals that might enter the release program to be vaccinated already, it is very unlikely that these animals could transmit the disease even if they were exposed.

Likelihood of Transmitting it to Others- Is the disease causing organism likely to be transmitted to other individual. This will be a function of many possible factors. The social behavior of the species may be significant in that primarily solitary species such as most big cats may be less likely to transmit diseases that require direct contact to each other than species that live in social groups such as many primates or hoofstock species. Another factor may be the organisms ability to survive in the environment. Many parasites survive well and remain potentially infective for a long period of time but many viruses do not.

Severity for the Individual- If an individual in the wild population does become clinically ill with the disease, how severe is it. Does it make the animal severely ill quickly and have a high probability of killing the animal or is it a disease that rarely kills an animal or takes a long time to have a significant effect.

Severity for the Population- If a disease is likely to spread quickly through a population and kill many animals in the population then it would be considered severe for the population as well as for individuals such as rabies in wild dogs or canine distemper in lions. However some diseases may not have a significant effect on individual animals but will profoundly effect the population such as the potential effect of brucellosis in wild cattle species which would not effect an individual animal in any significant way but could profoundly effect the wild population overall by reducing reproduction significantly.

Estimated Significance to the Program- Sum the numerical values assigned to each category for this particular disease. This value will give some sense of what the importance is of this particular disease for this release program and enable you to rank the diseases with regard to your intuitive sense of their importance. Diseases which have the highest values in this column will likely be the most significant diseases to address in sorting through the steps needed in a release program.

Disease__Canine Distemper, a viral disease primarily of dogs that causes CNS disease and possibly enteritis in big cats_____

Likelihood of Susceptibility___Based on known, documented outbreaks of canine distemper in captive populations in the United States tigers are known to be susceptible to the disease. Although research has not been possible to assign an accurate numerical value to the susceptibility of the species in general but it is reasonable to assume at this point that all individuals are highly susceptible. Vaccination is possible but very uncommon at this time._____

Likelihood of Exposure____The management of most captive animals makes it unlikely that they will be exposed to this virus which is normally transmitted by dogs and by raccoons_____

Likelihood of Becoming Infected Very little is understood about how this disease works in cats. I assume that it is possible that a carrier state can exist and that animals are likely to become infected if exposed._____

Likelihood of Transmitting it to Others Evidence from lions in the Serengetti indicates that this disease is highly infectious between individuals, however the generally solitary behavior of tigers in the wild makes it much less likely that they would transmit the virus to each other.

Severity for the Individual____Canine distemper has caused a high mortality rate in big cats both in captivity and in the wild____

_____

Severity for the Population____This virus seems to be potentially very serious for the wild population if it is introduced, having the possibility to eliminate many individual animals from the wild population_____

Estimated Significance to the Program This virus seems likely to be an important disease to investigate further and develop a management plan for for this program

References Used Appel, M.J.G., R.A. Yates, G.L. Foley, J.J. Bernstein, S. Santinelli, L.H. Spelman, L.D. Miller, L.H. Arp, M. Anderson, M. Barr, S. Pearce-Kelling and B.A. Summers. Canine distemper epizootic in lions, tigers and leopards in North America. 1994 J. of Vet. Diag. Invest. (In Press).

Roelke-Parker, M.E., L. Munson, C. Packer, et.al. A canine distemper virus epidemic in Serengeti lions (Panthera leo). Nature 379: 441-445, 1996

Disease__Toxascaris cati, an enteric parasite_____

Likelihood of Susceptibility__This parasite is quite common in captive animals and although most zoos treat for the parasite and it is possible to eliminate it , reinfection is quite common. Animals that are exposed are almost certainly susceptible to it._____

Likelihood of Exposure_____This parasite is quite common in captive animals and although most zoos treat for the parasite and it is possible to eliminate it , reinfection is quite common and most animals are likely to be exposed to it_____

Likelihood of Becoming Infected__The high probability of exposure and high susceptibility make it likely that animals will become infected_____

_____

Likelihood of Transmitting it to Others__Animals are very likely to shed oocytes of the parasite and transmit it to other cats. The solitary lifestyle of the animals reduces transmissiion likelihood somewhat

_____

Severity for the Individual_____Although heavy parasite loads can be debilitating, it is primarily an issue for very young or very old animals_____

Severity for the Population____Parasites probably will not have much effect on the wild population and are probably already present although that is not confirmed_____

_____

Estimated Significance to the Program_____The prevalence and susceptibility of the animals to this disease makes it likely to occur but it is of relatively low impact for the individual or the population. However it is also very straightforward to diagnose and to treat so it seems likely that it should be addressed in this program planning._____

References Used_____Fowler- Zoo Animal Medicine_____

Disease__Tuberculosis, Mycobacteria bovis. a bacterial systemic disease primarily of cattle but transmitted through meat or directly animal to animal primarily by aerosol_____

Likelihood of Susceptibility: Most mammals are considered resistant but susceptible in the right circumstances___

Likelihood of Exposure____The disease is very rare in most captive animal circumstances. Meat would pose the likeliest risk of exposure but most captive North American animals are fed inspected meat in which TB is very very rare. There have been cases in captive big cats fed meat from animals that died of TB and in wild lions in South Africa that feed on TB infected prey_____

Likelihood of Becoming Infected_Infection generally seems to require repeated, prolonged exposure and is very unlikely considering the source of the animals for the program._____

_____

Likelihood of Transmitting it to Others__The solitary behavior of tigers makes it very unlikely that any animal would be in sufficiently prolonged contact with another animal to be able to transmit the disease_____

_____

Severity for the Individual____Animals which develop clinical tuberculosis will be severely debilitated and die of the disease_____

Severity for the Population_____It is difficult to imagine a scenario where this disease could significantly effect the wild population of this species unless prey species were widely infected_____

Estimated Significance to the Program_____This disease is probably not of significant concern in this program

---

References Used_____Montali- Mycobacteria in Zoo Animals?; Fowler – Zoo Animal Medicine; Douw Grobler, South Africa, personal communication _____

Disease__Rabies, a viral disease causing central nervous system disease in mammals_____

Likelihood of Susceptibility____All mammals are considered susceptible if properly exposed to the disease, many zoos vaccinate for the virus with killed vaccines which is thought to protect the animals from the disease if exposed_____

_____

Likelihood of Exposure____Rabies does occur in North America in wild and domestic animals, however transmission is almost always directly from a bite from an infected animal, which is very unlikely to happen with this species_____

_____

Likelihood of Becoming Infected__The chance of even being exposed is very low and if vaccinated, it is very unlikely that an animal could develop the disease_____

Likelihood of Transmitting it to Others__Animals must develop clinical rabies in order to transmit it and then must bite another animal in order to transmit the disease. There is low probability that any of the introduced animals could develop the disease and if they did, it is unlikely they would encounter another cat to bite to transmit the disease to_____

_____

Severity for the Individual____Severely effects individuals, resulting in death in a relatively short time_____

Severity for the Population____Unlikely to have a significant effect on the wild population of tigers_____

Estimated Significance to the Program_____Unlikely important in this program but vaccination is relatively inexpensive and easy to do. Also it is assumed that a quarantine period of 2 weeks would result in any clinical cases being detected_____

References Used_____Personal knowledge of the disease_____

ANIMAL MOVEMENTS AND DISEASE RISK

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4. DECISION ANALYSIS PROTOCOLS

Protocol for Disease *Risk Analysis* for Animal Movement

***italicized words defined in glossary*

Section 1: Risk Analysis - Define the Big Picture Problem/Policy and Identify Potential Hazards

This is the area where you concentrate on the big picture. This is done in order to frame the issue in order to create a flow chart of the problem and identify potential hazards to be ranked in the hazard identification phase. Reintroduction of Ruffed Lemurs into Madagascar will be used as an example.

Step 1: Summarize the issues surrounding the entire process.
Provide background (tell the story) in introductory format

Example: Background on Lemur Movements

Project started 1995, involved North American and Betampona reserve in Madagascar (2500 ha East Coast – island pop) – carrying capacity of 60 (only 25-30 exist). Population modeling showed not sufficient genetic diversity to sustain long-term (however, can increase long-term genetic health if add 20+ animals). Move animals within Madagascar (but sub-speciation and behavioral abnormalities of pets a major concern). Therefore, decision made to move captive animals from zoos in N. American and European zoos. Interest to see if released zoo animals would work. SSP coordinator selects animals by SSP pedigree – only represented bloodlines, and would include extensive medical examinations; animals must have reproduced, be young adults (2-3 years) ideally (for long breeding life). The original plan was to release pairs or breeding groups. It is the responsibility of the veterinary advisor to look at medical concerns. In 1997 five lemurs were reintroduced, 1998 four, 5/9 are now dead. Pre-release training on St. Catherine's Island and Duke University – adds other disease risk issues. Released animals are radio-collared and tracked by field biologists. Ideally 6 month pre-release training, but actually 2-3 months, then another very short pre-release in reserve before actual release. Next release scheduled this fall (5 total, but one already died). Release during dry season – easier for biologists to track.

Potential Benefits of Animal Movement

Ideally, this program would allow roughed lemurs to reach carrying capacity and increase genetic diversity in the wild population for long-term genetic health. Lost 5 out of 9 already – not known if this rate is higher than natural occurrence. Some released animals have already integrated into natural groups; others are alone or are making new groupings. Two released pairs have reproduced, one reproduced with a wild lemur. Two surviving offspring have been produced from the translocated animals. Agricultural development around reserve (fragmentation) thought to cause original reduction in population. Last 10 years, increase in management of reserve, guards, increased research component, lots more activity and interest – seems to be secure at this time. Other benefits – flagship species to help protect reserve, and to continue interest in Madagascar and their own people and students. Two other projects this year

– research station (with a manager) initiated. Now other projects come in as a result. No ecotourism in this reserve. Permits available for research only.

Approached the task of evaluating disease risk by performing literature search and surveying animals in zoos in Madagascar to see major disease problems (found few). Added some more based on research and review.

Step 2: Identify and define potential problem(s)

What problem(s) does step 1 bring out that need to be evaluated?

- List all population-level problems. This is not yet the point at which you define the specific detailed risk assessment question so leave them broad. Take into account potential effects on the following species:
    - Humans
    - Domestic animals
    - Other wildlife species

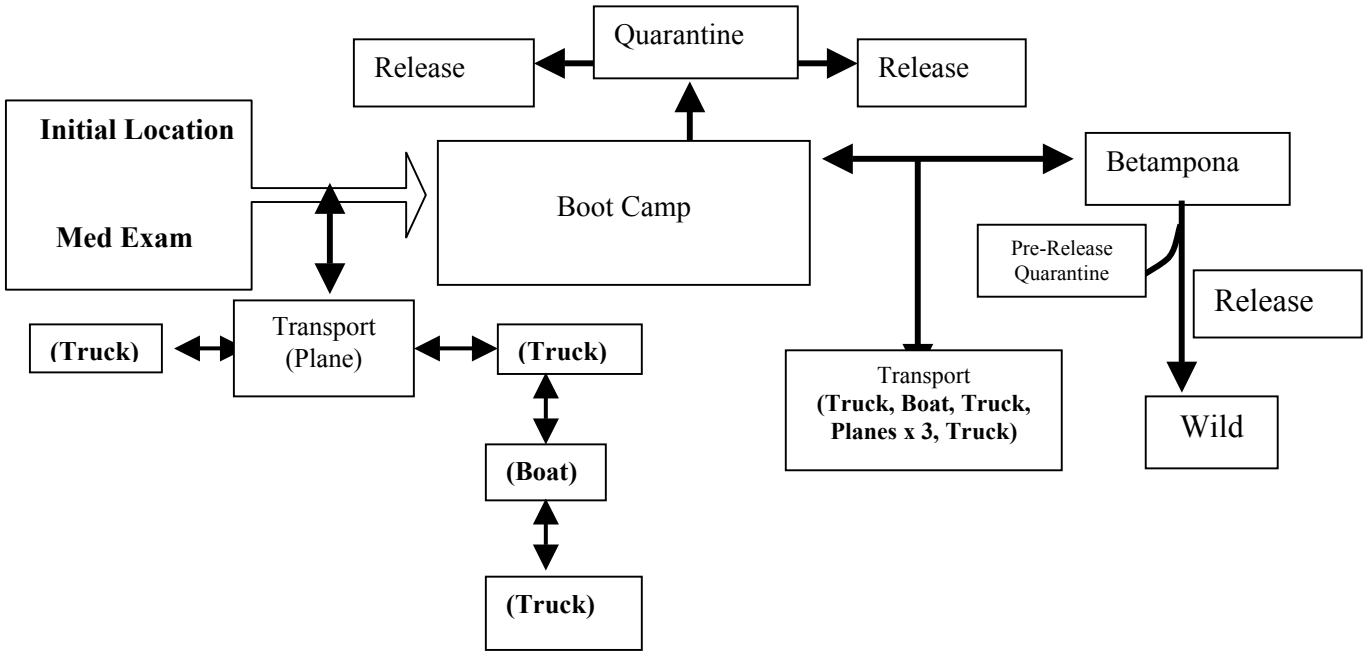
Example: Lemur disease risk

Disease may potentially impact this program in three ways. Reintroduced Lemurs may bring diseases with them that affect a naïve local free-range population. Naïve reintroduced Lemurs may succumb to existing diseases at the point of reintroduction. Disease may be introduced somewhere along the reintroduction pathway from human interaction, or be introduced to humans as well. Specifically, what is the likelihood (risk) of introducing a hazard into the Madagascan lemur population and to have it becomes endemic in the whole ecosystem (whole island)?

Step 3: Completely outline the general pathway.

- Detail all aspects of animal movements in box diagram form. This should diagram the entire flow of the process covered in the problem above.
- Include such things as
  - Source
  - Quarantine procedures
  - Transport methods
  - Procedures done at all points on diagram
  - End points
- Write a narrative for the flow diagram so that the pathway may stand-alone.

Example - Ruffed Lemur Reintroduction Pathway



Step 4: Hazard Identification - Identify and list all *potential hazards* (This will vary depending on the specific risk assessment).

- Create a master list(s) of diseases that potentially affect the species outlined in the problem. This information can come from sources such as:
    - PHVA
    - **Quarantine and health screening worksheet(s)**
    - Disease surveys
    - Literature search
    - SSP Veterinary Advisor protocols
- Identify potential hazards for each location (lists may vary depending on regional differences).
    - Source or point of origin
    - Midpoints along the pathway (i.e. during transport, handling, soft release, quarantine, etc.)
    - Destination population
- Make sure to consider all possible infectious and non-infectious disease processes including zoonoses that can be introduced along the pathway

Example - Ruffed Lemur Reintroduction

Diseases of concern:

Disease of Concern	Recommended Test	Where is it of concern	Testing Location	Sample Amount	Results
Hep A	Sero				
Hep B	Sero				
Herpes Simplex	Sero				
Cytomeg virus	Sero				
Epstein Barr	Sero				
Measles	Sero				
Salmonella	Fecal x 3				
Shigella	Fecal x 3				
Campylob	Fecal x 3				
Yersinia	Fecal x 3				
TB	ID skin				
Toxo	Sero				
T. cruz	Cult + Sero				
Cutarebra	P. Ex				
Strongyloides	Fecal x 3				
Entamoeba	Fecal x 3				
Lyme's	Skin biopsy				
Ehrlichia	Sero (PCR)				
RMSF	Sero (PCR)				
gEctos (ticks)	P Ex				

Step 5: Create list of *hazards*

- The point of this step is to filter the larger list of potential hazards down to those that need to be modeled in the risk assessment (hazard list). These are high risk diseases that need to be assessed in detail.
- Create hazard list from potential hazard list using *ranking criteria*
    - Define ranking criteria (determined by the risk assessor).
        - These are factors that are important in determining if potential hazards should be fully assessed in the *risk assessment* (i.e., potential hazards to *hazards*).
        - Example of ranking criteria include:
            - ***Infectivity***
            - ***Pathogenicity****:* Morbidity and mortality
            - ***Transmissibility****:* Routes and rates; Presence of competent vectors
            - ***Susceptibility****:* Species of concern; Source and destination; Humans; Domestic animals; Other wildlife species
            - **Severity in terms of**: Reproductive effects; Morbidity and mortality; None (or unknown); Immunosuppression (alter susceptibility);
            - **Economic impacts on**: Species of concern; Ecosystem; Humans; Domestic animals
            - **Existing prevalence and incidence**

- You should now have a list of hazards that is a subset of potential hazard list
- Each hazard must be assessed in the *Risk Assessment*.

Section 2: Risk Assessment

A risk assessment must be done on each hazard identified in section #1. Risk assessment is the process of determining the likelihood or probability of adverse health effects associated with hazard exposure. This may be qualitative or quantitative depending on the needs of the users and the amount of data available. At this point, the problem definition needs to be very specifically refined.

Step 6: Define specific risk assessment question.
In order to build a model, a specific question must be asked. This is usually one of many questions that could be asked under the broad policy question. This question will be asked for every disease of concern identified in the hazard identification phase above.

Formulate a specific question including all or some of the following:

- Species
- Source
- Destination
- Specific hazard(s)
- Transport method(s)
- Pathway

Specific question format: What is the likelihood of introducing [species, animal or group] positive for ["x" hazard] from [source] to [destination] via [transport method] on [pathway]?

i.e. *What is the likelihood of introducing Lemurs positive for TB from Kalamazoo to Timbuktu via the transport route described in the pathway flowchart for reintroduction?*

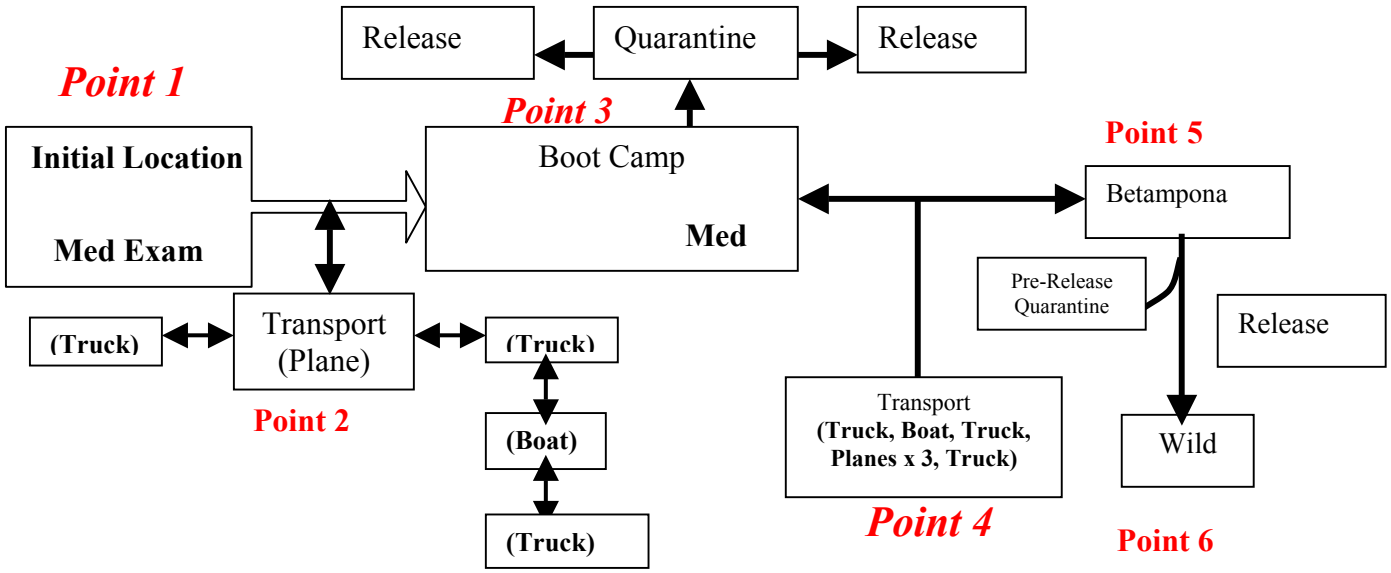
Specific Example: Ruffed Lemur Reintroduction

What is the likelihood of introducing TB into lemurs into Betampona given that the current population is TB-free?

Step 7: Build the Risk Assessment Model to Assess Hazard

- Draw a flow diagram based on the specific question (this may be identical to the general flow diagram in section 1, step 3 or may include additional steps specific to the narrow question).
- Identify important steps known as critical control points (CCP). A critical control point is any point in the transportation pathway where the hazard may be introduced or released (depending on the question) into or from the pathway. These are subjective and will vary depending upon case scenario.

Example: Ruffed Lemur Reintroduction



Step 8: Perform Qualitative Assessment

- Define qualitative grading scale – examples include high/medium/low; present/absent; yes/no; acceptable/non-acceptable.

- For each CCP, describe the important factors that contribute to the risk at that point. For instance, the prevalence of disease in the source population (high/medium/low), the sensitivity of diagnostic tests (good/bad), the likelihood of a disease being introduced through human contact (high/medium/low).

- State the *assumptions* used in the model. Assumptions are the conditions surrounding critical control points that need to be clarified for the process to be repeatable. For example, when including animal shipment as a CCP, you need to define the details of the move such as the type of container, how many animals will be held in the container, the kind of vehicle used etc.

- Assess the integrity of the model

- Does it make sense?
    - Does it flow?
    - Are there too many steps?
    - Are there not enough steps?

- Does it answer the question?
    - ***If yes****, congratulations – you have completed your Risk Assessment!*
    - ***If no****, move on to Step 9 (Quantitative Assessment).*

Example: Ruffed Lemur Reintroduction

Model Formulation:

Point 1: Probability of animal x leaving the zoo infected with disease y
Probability of an infected animal existing at the zoo = LOW
Probability of not detecting (FN) before it moves on = LOW
OVERALL = LOW

Point 2: Probability of previous infection surviving transport or introduction of agent during transport.
Probability of host survival
Probability of agent survival
Probability of introduction during transport
Assume that no agent introduced and agent survives so overall remains LOW

Point 3: Probability that infectious animal gets out of boot camp.
Prob. of FN on initial exam/quar = LOW
Prob of FN on final quar exam = LOW
Prob of introduction of new infection and FN test = VERY LOW
OVERALL = LOW - VERY LOW

Point 4: Transport #2: Probability of previous infection surviving transport or introduction of agent during transport from GA to Madagascar.
Probability of host survival
Probability of agent survival
Probability of introduction during transport
Assume that no agent introduced and agent survives so overall remains LOW - VERY LOW

Point 5: Probability of release in Madagascar at release site (no testing) or introduction of agent before release.
No risk was greater than LOW so overall cumulative is LOW

Step 9: Perform Quantitative Assessment <u>if Needed</u>

There are numerous ways to do this depending on the amount and quality of the data. This may be done deterministically or stochastically. Deterministic models use point estimates for the variables while stochastic models use more complicated formulas to incorporate variability. Deterministic models depend on things such as means and medians while ranges, standard deviations or variance estimates, may represent stochastic variables. Deterministic quantitative assessments may be done using paper, pencil and a calculator, as long as the person has a good background in basic probability theory. Stochastic models make use of more complicated software; for these projects, it is recommended to consult a risk assessment specialist.

Deterministic model

- May be used when reliable point estimates are available and there is little uncertainty or variability surrounding the data. Can also be used when little data exists and expert opinion needs to be relied upon.
    - Point estimates can be:
        - Number(s), mean, std. deviation etc.
        - Probability
        - Percentages
    - Derive estimates from: Literature; Expert opinion (personal communication); Personal experience; Specific data
        - [Try to avoid guessing if possible – but, sometimes that is all that is available. The lack of information should be described under uncertainty and may also help to guide future research resources and efforts.]
    - Multiply point estimates for final risk probability.
        - Did it work?
        - Does it make sense?
        - Does it answer the question?

<u>Example: Ruffed Lemur Reintroduction</u>

Deterministic Model Formulation:

Point 1: Probability of animal x leaving the zoo infected with disease y
Probability of an infected animal multiplied by:
Probability of not detecting (FN) before it moves on (1-Se)
Can assume an infection (1) or use prevalence estimate
[Skin test and radiograph, CBC and physical exam for cumulative sensitivity of 0.5]
Prev est = 0.001 (3pos/5000 animals in 25 years)
1-.5 = .5 (p)FN
.001 x .5 = .0005 = likelihood of infected animal leaving zoo with TB

Point 2: Probability of previous infection surviving transport or introduction of agent during transport.

Probability of host survival
Probability of agent survival
Probability of introduction during transport
Assume that no agent introduced and agent survives so prob still .0005

Point 3: Probability that infectious animal gets out of boot camp.

Prob. of FN on initial exam/quar
Prob of FN on final quar exam
Prob of introduction of new infection and FN test
2 tests X .5 p(FN) = 0.25
.0005 X .25 = .000125 chance that animal gets out of boot camp with infection

Point 4: Transport #2: Probability of previous infection surviving transport or introduction of agent during transport from GA to Madagascar.

Probability of host survival
Probability of agent survival
Probability of introduction during transport
Assume that no agent introduced and agent survives so still .000125

Point 5: Probability of release in Madagascar at release site (no testing) or introduction of agent before release.

0.0001 (1 in 10,000 animals) chance of releasing a positive (TB) Lemur into wild based on human prevalence and wild (guestimate)

CONCLUSIONS:

As a result of low likelihood of release, both qualitatively and quantitatively, the decision was made not to pursue further studies using stochastic modeling.

What if I need to get fancier??? → Stochastic model

- Used to incorporate uncertainty surrounding point estimates. This adds a level of complexity to the model and will need to be performed with the assistance of an expert modeler.
- Moving from point estimates to the incorporation of ranges or variability in the model. Rarely does a point estimate actually represent the true likelihood of an event. Stochastic modeling allows for variability of the estimate to be incorporated into the model.
- Advantages of using it?
  - Incorporating the use of distributions (e.g., worse versus best scenarios)
  - Easily adaptable/changeable
  - Can perform multiple scenario
  - More rapid, once set
  - Sensitivity analysis
  - Simulations/Monte Carlo

- When is it needed?
    - More detailed knowledge
    - Increases credibility
    - Have to use a range for estimate
    - Have expertise, funds and time
    - Seriousness, complexity of the problem

- Useful tools (e.g., software)?
    - Excel
    - @Risk or Decision Tools (Palisade Co.)
    - Stella
    - Vortex (CBSG)
    - Epi Info (CDC)

Step 9: Describe *uncertainty* of process.

Describe all of the things that you are unsure about in this process. Also describe the degree to which you are unsure. Areas usually included are: the pathway flow diagram, the CCP's used in the model, the data inputs (point estimates etc.), Sensitivity/Specificity of diagnostic tests, disease prevalence estimates, host and agent survivability and transmission efficiency.

Glossary:

Acceptable risk: Risk level judged to be compatible with the protection of animal and public health within the pathway of concern.

Assumptions: Properties/characteristics of parameters in a risk assessment which are fixed within the model and do not change. They may be objective or subjective, but must be explicitly stated in the risk assessment to enhance transparency and risk communication.

Deterministic model: A model whose inputs are completely determined by a given set of conditions resulting in point estimates.

Hazard: A potential hazard that meets the specifications of established ranking criteria and is now considered a high priority potential hazard; all identified hazards must be included in the risk assessment.

Hazard identification: The process of identifying the pathogenic agents which could potentially be introduced into or released from the reintroduction pathway of concern.

Infectiousness: The ease by which a disease organism is transmitted from one host to another; often used synonymously with transmissibility/communicability.

Infectivity: The characteristic of a microorganism that allows it to infect and subsequently survive and multiply within a susceptible host.

Model: Diagram, flow chart, mathematical or statistical summarization/representation of a complex real-world process.

Pathogenicity: Host-specific ability of an agent to cause disease or otherwise induce pathological change in a susceptible host.

Potential hazard: Any pathogenic agent that could produce adverse consequences on the reintroduction program.

Qualitative risk assessment: An assessment where the outputs on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as high, medium, low or negligible.

Quantitative risk assessment: An assessment where the outputs of the risk assessment are expressed numerically.

Ranking criteria: Specific characteristics, properties or attributes of an agent or situation used to differentiate a potential hazard from a hazard during hazard identification; criteria used to decide which potential hazards must be assessed in the risk assessment.

Risk: The likelihood (probability or frequency) and magnitude of the occurrence of an adverse event or hazard; a measure of the probability of harm and the severity of the unwanted adverse effect.

Risk analysis: The process composed of hazard identification, risk assessment, risk management and risk communication.

Risk assessment: The evaluation of the likelihood and consequences of entry, establishment, or spread of a pathogenic agent within the pathway or species of concern.

Risk communication: Risk communication is the interactive exchange of information on risk among risk assessors, risk managers and other interested parties (stakeholders).

Risk management: The process of identifying, selecting and implementing measures that can be applied to reduce the level of risk.

Sensitivity analysis: The process of examining the impact of the variation in individual model inputs on the model outputs in a quantitative risk assessment.

Stochastic/probabilistic model: A model whose inputs represent the inherent variability and uncertainty of the situation; this may be accomplished by incorporating variance and standard deviations around point estimates or by performing multiple iterations of the model using a random number generator.

Susceptibility: The state of being readily affected by a pathogen; a lack of resistance to a pathogen.

Uncertainty: The lack of precise knowledge of the input values which is due to measurement error or to lack of knowledge. The degree to which you don't know the answer to a specific question. For example, the sensitivity or specificity of a radiograph for diagnosing TB is very uncertain.

Variability: A real-world complexity in which the value of an input is not the same for each case due to natural diversity in a given population.

Virulence: The host-specific ability of an infectious agent to multiply in the host while inducing lesions and disease.

Glossary References:

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ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

5. SYSTEM MODELING - STELLA AND VENSIM

Using Simulation Models to Assess Effects of Disease (and Other Things!) on Populations

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One of the most difficult challenges is making decisions when information about the decision process or possible outcomes is uncertain. When assessing risk of animal movements, there is never likely to be complete data and there are a number of different possible outcomes that are also unknown. One technique for trying to improve decision-making is to use existing scientific data and estimates about the likelihood that certain things will happen to create a model of the situation and predict outcomes. This approach to decision-making has already become wide spread in other fields, and continues to grow as a method for dealing with complicated human and animal health issues. Modeling infectious disease helps us conceptualize and summarize the risk of disease introduction and transmission.

One advantage of modeling is that it creates an explicit, visual picture of our current beliefs and understanding about a problem. If we use modeling software, like STELLA® or Vensim®, to compose this picture, we can then simulate and predict the logical outcomes from this vision. If these results don't match field observations, it shows us that either our model needs to be revised or that our real world data are biased. The conceptual model may highlight critical information that is currently unknown and needs to be collected before solving the problem. Sensitivity analysis of the model identifies the factors that most strongly influence outcomes. If very contentious points have little impact on final outcomes, this can help build consensus. Models can, additionally, be used to predict consequences, compare potential programs or policies, and quantify efficacy of interventions. Prediction of consequences and evaluation of the effectiveness of interventions are major goals of disease risk assessment.

The reason we are now seeing so many risk assessment and other types of computer models is that they provide a way to address issues that are perceived as "imminent threats". You can't generally know, from past experience, **exactly** what will happen in the future, especially if you are considering doing something that has never been done before. You can use existing data to try to predict, but you can't know for sure until it happens. Models provide a way of making educated predictions resulting in decisions when there is uncertainty. They are seen as these magic "black boxes" that give us answers.

Modeling and risk assessment can be accomplished mentally or using pencil and paper. Computer programs are useful tools as problems or potential options grow more complex. There are a number of different computer programs that can facilitate this process. STELLA® is a commercial software program designed for modeling complex problems, made by High Performance Systems, Inc. Information is available at the website: http://www.hps_inc.com/edu/stella/stella.htm. Vensim® is another commercial software package, that offers the advantage of a free, scaled down version. Information is available at the website: http://www.vensim.com/ Both are graphically oriented programs, which allow diagrams of the problem to be made, then the underlying equations to be completed, then the outcomes to be simulated, and parameters varied. Because they are simple to begin using and have this

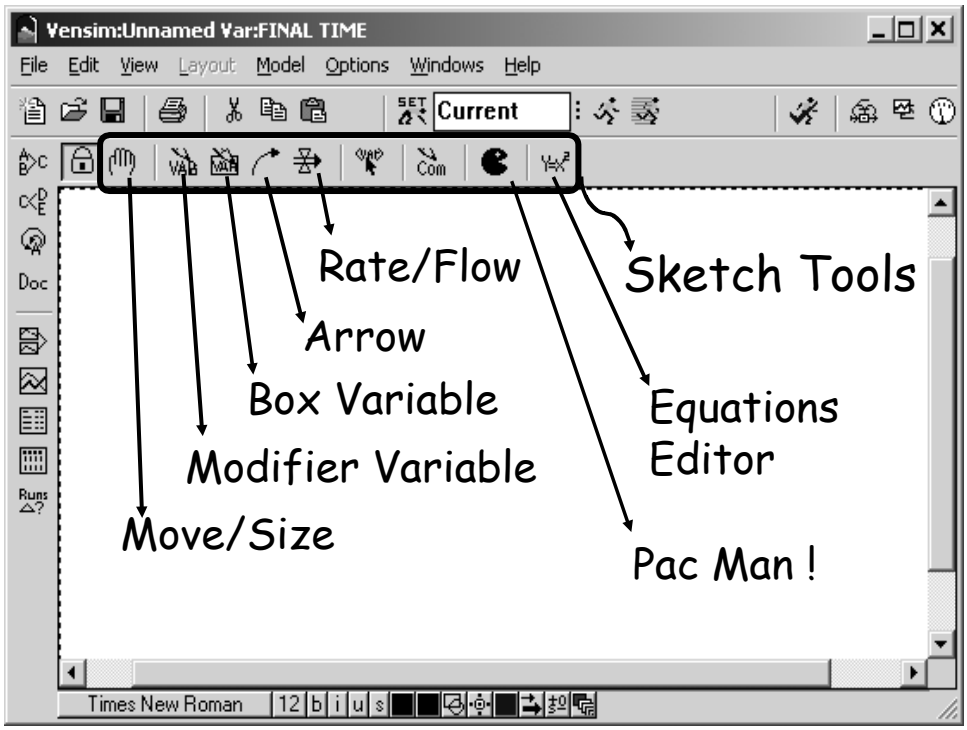
graphical interface, these programs lend themselves very well to modeling problems in groups of experts from diverse fields.

A challenge in constructing a model, using either program, is finding the appropriate data. Sources may include the scientific literature, field studies, epidemiologic analyses of risk factors, best guesses, etc. Accepting the validity of the data and agreeing on the underlying assumptions is often the most contentious step in the modeling process. Recognition of specific new data items that need to be collected is a common outcome of the modeling process.

Although STELLA® and Vensim® are very powerful, they each use a simple set of tools that can be learned very quickly. The following example will show how the tools might be used in building a Vensim® model of disease transmission.

There are two main sets of tools in Vensim. The Sketch Tools are used to create a model and the Analysis Tools are used to conduct diagnostics on the model and to view output.

Sketch Tools



Box Variables are used to hold or accumulate numbers of things. In disease modeling, these are usually numbers of animals in different stages of disease. Susceptible, infected, and immune subpopulations would be examples of potential boxes in a model. Animals can be thought of as physically moving from one box to another, or they can be considered to have "moved" if their characteristics change, as with disease models.

Rates, or flows are used to model movement between boxes. A rate arrow would allow susceptible animals to become infected at some specified rate. This flow would cause the number of susceptible animals to decrease and the number of infected animals to increase.

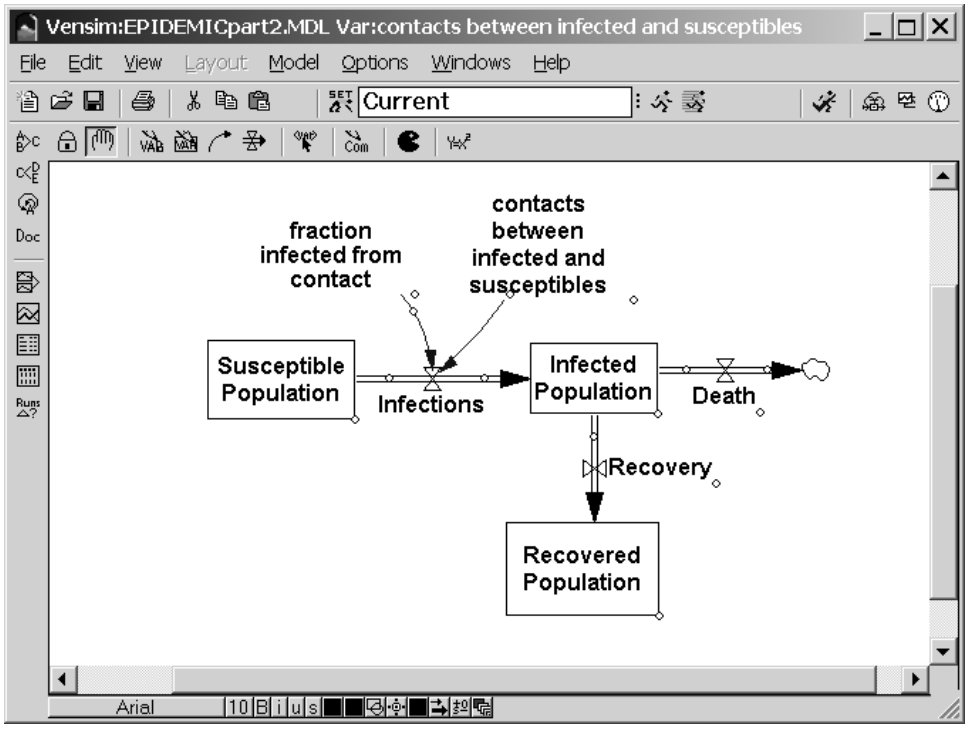
Modifier Variables hold information that stays constant or that is needed to modify the flows in the model. These are a convenient way to represent data that are not the actual numbers of animals, but affect the way animals move between the subpopulations in the model.

Arrows illustrate links between parts of the model, aside from the movements of animals. All of the factors that go into calculating the rate at which animals move between stocks are linked to the flow rate calculation through arrows.

Move/Size Hand is used to select, stretch and move variables, arrows, rates, etc. when building a model.

Pacman is used to "gobble-up" unwanted components when building a model. It is equivalent to the delete key in other software programs

An example of a simple epidemic model would be boxes for susceptible, infected and immune animals with flows between them for infection, recovery and death. This captures, conceptually, the bare bones of the dynamics of an infectious agent in a population.



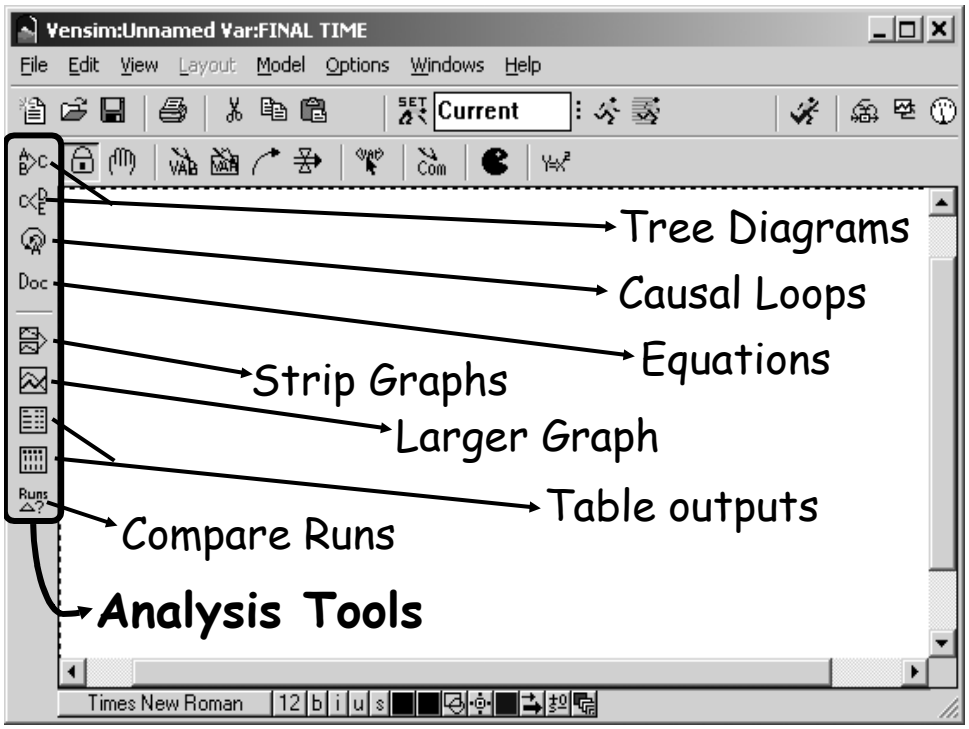
Starting from this type of base model, additions and enhancements, specific to a disease can be added to make the model more realistic. The animal population dynamics and potential preventive measures can also be included.

This visual representation is the first step in creating a quantitative model that can generate numerical predictions about disease patterns, transmission and risk. But, in many cases, just the process of specifying the model gives insights. It provides a visual summary of what we believe the relationships to be within a complex situation. This can allow us to recognize relationships that were not previously apparent and also stimulate discussion about the problem being modeled between people from disparate backgrounds.

Once the basic structure of the model has been constructed, the **Equations Tool** can be used to click on a variable or flow to open a window where values, relationships and equations can be defined. Data for this aspect of the model can come from review of the scientific literature, field studies, epidemiologic studies, expert opinion, and modeling short-cuts which produce a specific pattern. If some of these data are less than satisfactory, they can be modified later to substitute other values and see if the model predictions are sensitive to these changes.

Entering data and equations moves the model from a visual, conceptual tool, to an analytical tool that can be used to make predictions about the patterns of disease under different scenarios. Vensim provides a number of **analysis tools** for first checking the logic and links within the model (Tree diagrams, causal loops, summary of all equations), and then for visualizing, tabulating and comparing results from running simulations (graphs, tables and comparing data from different runs).

Analysis Tools



Causes and Uses Trees produce tree diagrams of the causal relationships for a particular variable, showing either the variables that feed into it (Causes) or those that it contributes to (Uses).

Loops gives information on the number of loops that pass through the selected variable.

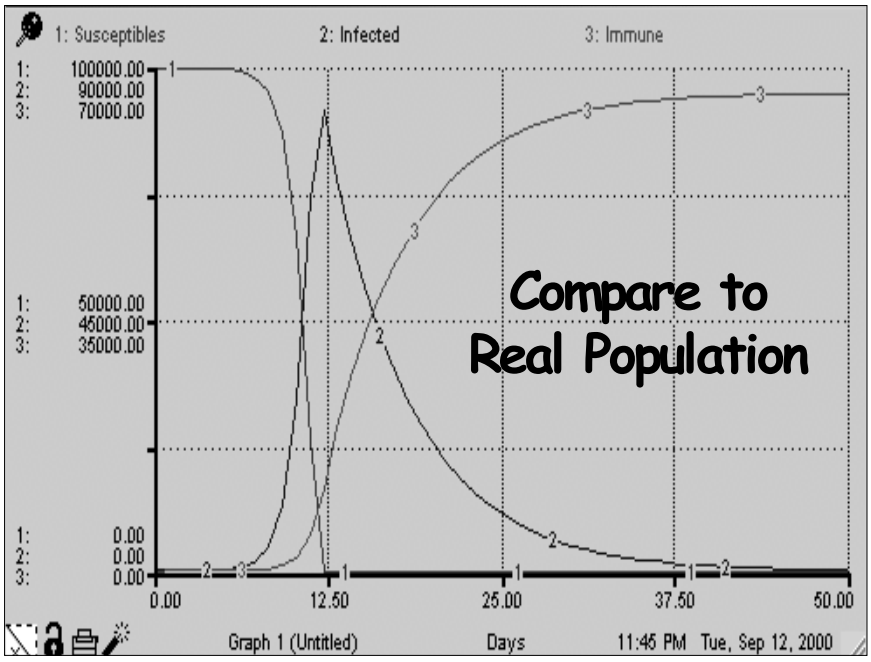
Document shows the equations currently defined for the entire mode.

Causes Graphs shows small graphs of the results of a simulation for a selected variable and the other variables in its causal tree, together in a window.

Graph displays a larger graph of results for a selected variable (The control panel can be used to create custom graphs of any variables).

Tables provides the results of a simulation in a spreadsheet-like format.

These tools facilitate the final steps in creating a model: verifying and validating. Verifying includes careful assessment of correctness of the relationships and numbers in the model. An especially nice feature of Vensim is that you can view tiny graphs within each box variable to show how the population size changes as the model runs. This allows visual trouble-shooting. Models are then validated by generating predictions and comparing them to actual data to see how well the model mimics reality. If predictions and reality are far apart, this may illustrate a gap in our knowledge about the problem and lead to modifications of the model. Importantly, if the model is the logical expression of our understanding of the system and it doesn't lead to realistic conclusions, then our view of the problem may need to be adjusted.



STELLA®, Vensim® or other similar modeling programs can help us visualize a problem for discussion, quantify relationships, and generate predictions. We can link together and make explicit what we know, believe and perceive about a problem. This provides a valuable tool for addressing complex risk assessment problems, comparing alternative actions and aiding decision-making. The ease of use allows content experts, intimately involved with the problem, to create and modify models rather than to rely on external modeling specialists.

Creating Your First Vensim® Epidemic Model

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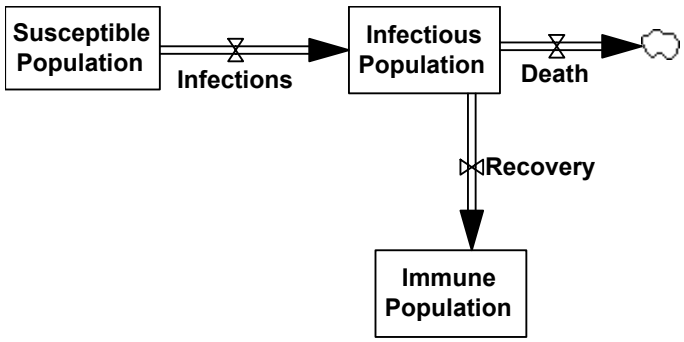
Modeling and risk assessment can be accomplished mentally, using pencil and paper, or with a computer. Software programs are useful tools for tackling more complex problems or those with more potential options for intervention. Modeling programs also provide a means to go beyond drawing conceptual pictures to explore relationships through simulation. Vensim® is free commercial software package, from Ventana Systems, Inc. There is also a more sophisticated version of Vensim® that has a cost. Information is available at the website: http://www.vensim.com/

Vensim® is a graphically oriented program. This allows a schematic diagram of the problem to be made, then the underlying equations to be filled in, and then a simulation run to generate predicted outcomes. It is easy to change values of inputs, both between and during runs, to see the effects of these changes produce on the outcomes. STELLA® is another similar commercial software program (but without a free version), made by High Performance Systems, Inc. (website: http://www.hps_inc.com/edu/stella/stella.htm). Because these programs are simple to begin using and have a graphical interface, they lend themselves very well to modeling problems in groups composed of experts from diverse fields.

We have two examples models for you to use as practice: a model of the deer population on the Kaibab Plateau (courtesy of Alberto Paras) and a simple model of an epidemic courtesy of Laura Hungerford and Carmel Smith). General directions for Vensim® are also included in the workbook. Additionally, the entire Vensim® manual is available through the **help** function when you are in the modeling program.

A first step in modeling disease dynamics (once you have developed a specific hypothesis and a conceptual idea about your system!!!) might be to draw a simple SIR (Susceptible-Infectious-Recovered) epidemic model. We will use an example epidemic model to both introduce and practice Vensim® and to provide a starting point for customizing your own model for a particular disease.

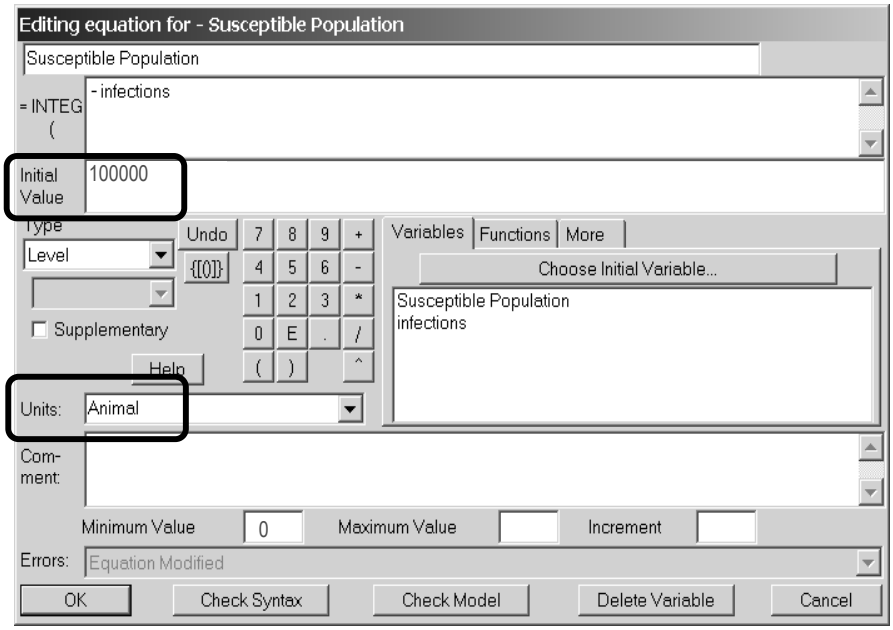
To create the basic epidemic model, first, draw the boxes shown below to represent the 3 states of animals in the population: Susceptibles, Infectious, and Immunes.



To make this model, first click on the **box tool**, then click on the diagram. When the typing symbol appears, type in the name of the box (for example, susceptible population). Then, click elsewhere on the diagram and repeat the process to create two other box variables, or states (for this example, infectious population and immune population). Next, draw "flows" to connect the boxes. Flows are used, rather than arrows, to show that animals can change or move from one state to another. Click on the **flow tool**, then on the box where you'd like the flow to start. Move to the box where you'd like the flow to end and click on this box. Then, type the name of the flow. To create a flow that represents mortality, click on the infectious box and then click on the diagram outside the box.

This creates a basic conceptual picture of the disease dynamics. This model shows that animals can be susceptible, then become infectious and recover to become immune or they can die. At this point, we can go on to either use some basic knowledge about disease transmission to define how susceptible animals move to the infectious box, or we can continue adding risk factors and modifiers to our vision of the disease to make the model more complex.

Let's begin by adding some of data that are pretty straightforward. As a starting point, we will define the initial population as having 100,000 susceptible animals, one infectious animal and no immune animals. These are nice round numbers, but it would be even better to put in the sizes from a real, known population. To enter these values, click on the **equations symbol**, and then click on the susceptible population box. A popup window will appear that is titled: **Editing equation for – Susceptible Population**. In the space to the right of the words **Initial Value**, type 100000, then in the **Units** space, type Animal. Click OK to close the window. Repeat this process, clicking on the other two population boxes to define the starting population size of 1 for the infectious population and 0 for the immune population.

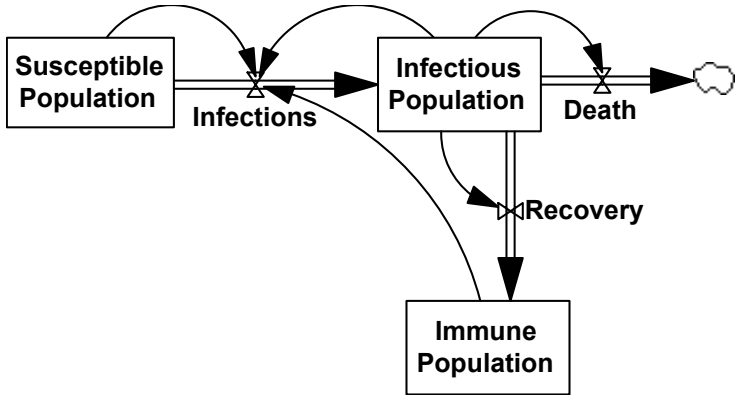


Now open the susceptible population box, again. You can see that Vensim® has already begun to define relationships based on our picture, since the **= INTEG** space has been automatically filled in with – infections, showing that the susceptible population will decrease from one time period to the next by the number of animals that become infectious. There are a number of other

options on this screen that you can read about in the manual (to access the manual, click on **Help**). Click OK to close the window.

For a simple, directly transmitted infectious disease, new cases occur when an infectious animal that is shedding the agent comes in contact with a susceptible animal and the agent successfully infects the susceptible animal (actually, we all know that infectious diseases are much more complicated than this, but we can add complexity later). To draw the relationship just described, we use the **arrow tool**. Click on the **arrow tool**, and then click once on infectious population, followed by a click on the flow labeled infections. The **Size Hand tool** can be used to change the shape and size of the arrow. This shows, graphically, that the number of infectious animals will affect the number of new infections that occur. Arrows are used when you want to show that there is a relationship between two variables, as in this case where the number of infectious animals partially determines the number of new infections. Flows are used to show that animals "move" through the connection, i.e. susceptibles move from being susceptible to being infectious.

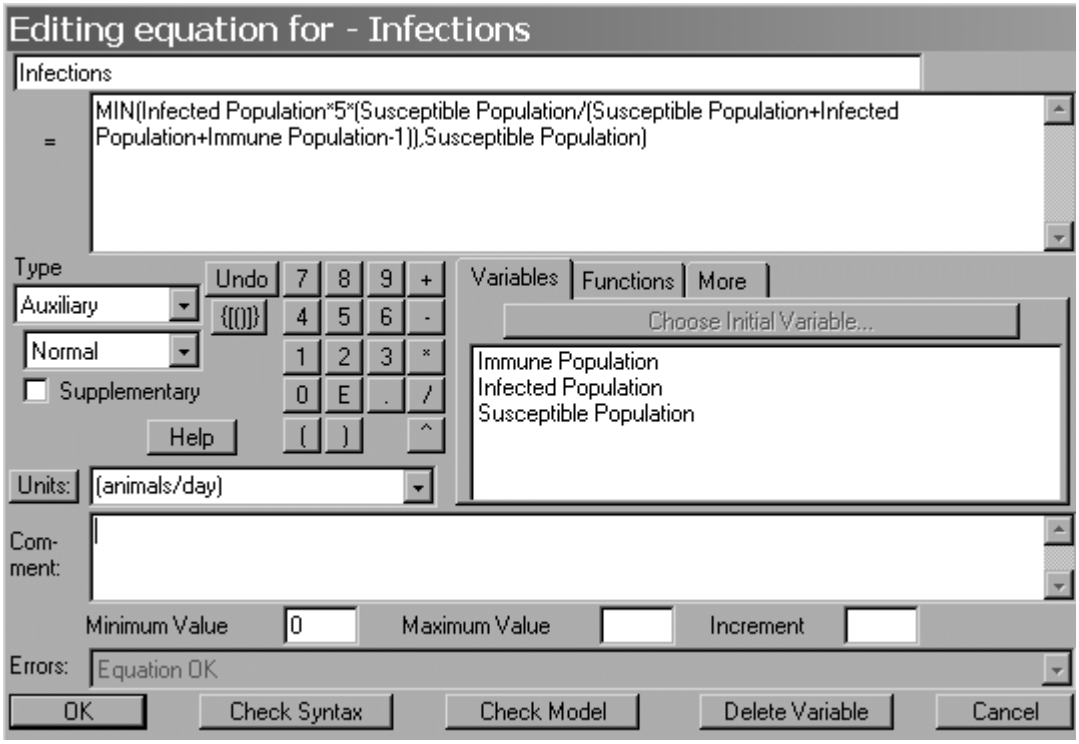
Draw another arrow, this time from the susceptible population box to the flow labeled infections. This shows that the number of infections will also be affected by the number of susceptibles that we have. But this leaves out the effect that the immunes have on disease dynamics. If an infectious animal comes into contact with an immune animal, instead of a susceptible animal, no new case will occur. This is the principle behind herd immunity. To add this relationship, draw an arrow from the immune population box to the flow labeled infections. You can also draw arrows from the infectious population to the death flow and to the recovery flow. This shows that the number of animals that recover or die depends on the number that have the infection.



Now, we're ready to start writing equations to describe the rates of infection, recovery and mortality. Eventually, we'd want to add more complexity to the way we draw and define this model. We want people to be able to look at the picture of our disease and get a complete idea of our theories about the factors that cause animals to become infectious, recover or die. This means that if we think that nutritional status or age or animal density or other factors affect the likelihood that an animal becomes infected or dies, we should add these factors to our drawing and link them to the flows with arrows. We can come back later and add variables to our diagram to show other relationships that affect transmission.

Use the **equation tool** to click on the label of the infections flow. This opens a pop-up window titled **Editing equation for – Infections**, to help us write the equation. We can enter an equation

to tell the number of susceptible animals that become infectious each day in the large space to the right of the equal sign. Through the diagram that we made, we've already specified that the equation should include the number of infectious, susceptible and immune animals, because we drew arrows from these state variables to the flow. Because of these arrows, Vensim® has already listed these states (Immune Population, Infectious Population, Susceptible Population) in the box below the **Variables** tab.



Let's assume for a start that for this disease in this species, each infectious animal will come in contact with 5 other animals each day and be able to infect them if they are susceptible. To define this relationship, first click on the words, Infectious Population, below the **Variables** tab. This will cause Infectious Population to appear in the Equation space to the right of the equal sign. Then, click on *, then 5. You can either use the number pad in the middle of the window or the keyboard. This creates an equation that gives the number of possible infectious contacts (and thus new cases) each day, based on the number of infectious animals. Since we used an equation, the number of contacts will change over time as the number of infectious animals changes. But we know that as the epidemic goes along, each infectious animal will encounter 5 other animals each day.

If we stopped here, each infectious animal would produce 5 new cases, regardless of the size of the total population or the number of susceptible animals. This would work for a huge population and a disease that didn't produce any protective immunity. But normally, after a while, at least some of the animals that the infectious animals bump into will already be immune. Or, the population will just run out of susceptibles because everyone has become infected. In this case, contacts will not produce new infections. So, the number of new cases per day has to be adjusted to show the proportion of contacts by infectious animals that are with susceptible

animals. This proportion is just the number of susceptibles (Susceptible Population) divided by the total number of animals in the population , minus 1, since an infectious animal can't really come into contact with itself (Susceptible Population + Infectious Population + Immune Population -1) .

To add this to the equation, we click on the *, then the (, then the following: Susceptible Population, /, (, Susceptible Population, +, Immune Population, +, Infectious Population, -, 1),). The reason to include the parentheses is to group together the variables that should be added together before they are divided or multipled. Vensim® will thus do the addition within the inner parentheses first, then the division within the next and finally the multiplication outside the parentheses. Although you could leave out some of these, it is good to add them to keep it very clear which parts of the equation are grouped together. This results in the equation shown above in the diagram.

Additionally, we need to specify what the program should do when there are not enough individuals left in the susceptible population to create the fraction that the above equation specifies. To do this click on the **Functions** tab in the Equations Editor box for Infections. A drop down list several function options are available. Select the MIN function and insert it at the beginning of the entire equation using the cursor. Right after the MIN function, insert (. At the very end of the equation, add , Susceptible Population) by selecting it from the variable box or typing it into the equation. The completed equation specifies that if the equation becomes larger than the numbers of animals in the Susceptible Population, then Vensim should just move the rest of the individuals from the Susceptible Population into the Infected Population Group. In other words, you can't move more individuals into the Infected Population than are available in the Susceptible Population. The final equation should look like this:

MIN(Infectious Population*5*(Susceptible Population/(Susceptible Population+Infectious +Immune Population-1),Susceptible Population)

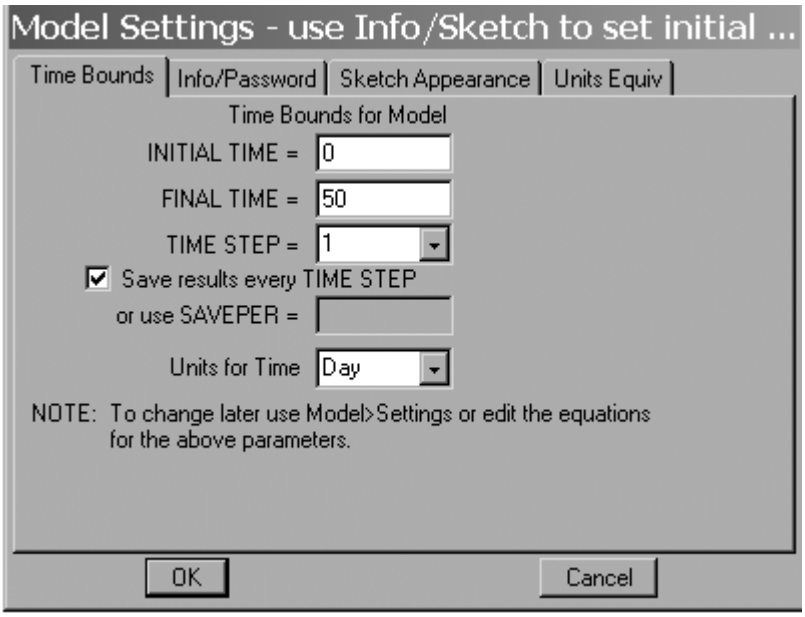
This is a very simple form of the equation and there are a number of other ways that the equation could be modified to make it more realistic. But this gives a good starting place.

The last step is to set the units to animals/day. Put the cursor in space to the right of the word **Units** and then type animals/day. Then click on OK to close the box. If you have a typo, it will give you an error message. Otherwise, the box should disappear. That takes care of the flow that defines how susceptibles become infectious.

To define rates for the last two flows, click on the **equation editor** and then the word death in the flow. The popup window appears for entering the equation and the variables list shows that, based on our drawing, we believe that the number of infectious animals will determine this flow. If we think that 5% (expressed as 5/100 or 0.05) of the infectious animals that are in the infectious group at that moment will die each day, we can write this equation as MAX (Infectious Population * 0.05 , 0) . Again, we use the function MAX to specify that if the Infectious Population multiplied by 0.05 gets smaller than 0, to return 0 animals instead of a smaller fraction of animals. Define the units as animals/day. Close this window and then use the **equation editor** to enter an equation for recovery as MAX (Infectious Population * 0.1 , 0) and change the units to animals/day. This means that 10% (expressed as 10/100 or 0.1) of the infectious animals that are in the infectious group at that moment will recover each day. Then click on OK to close this window. These values are just a start and you can change them to see what will happen to the epidemic curve.

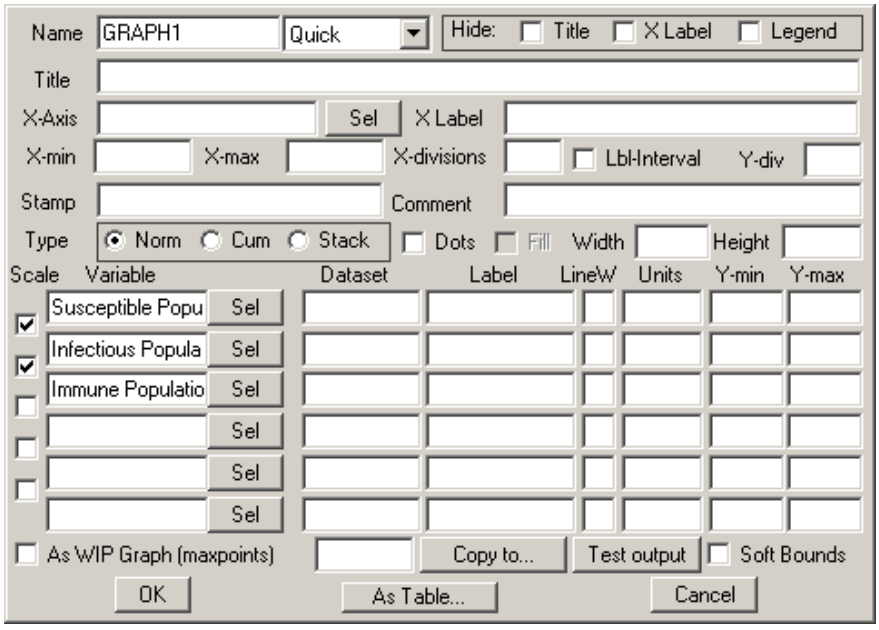
Now, there should no longer be any words or boxes that are highlighted in black when you click on the **equation editor**. The black highlighting is to show any variables or flows that have not been defined.

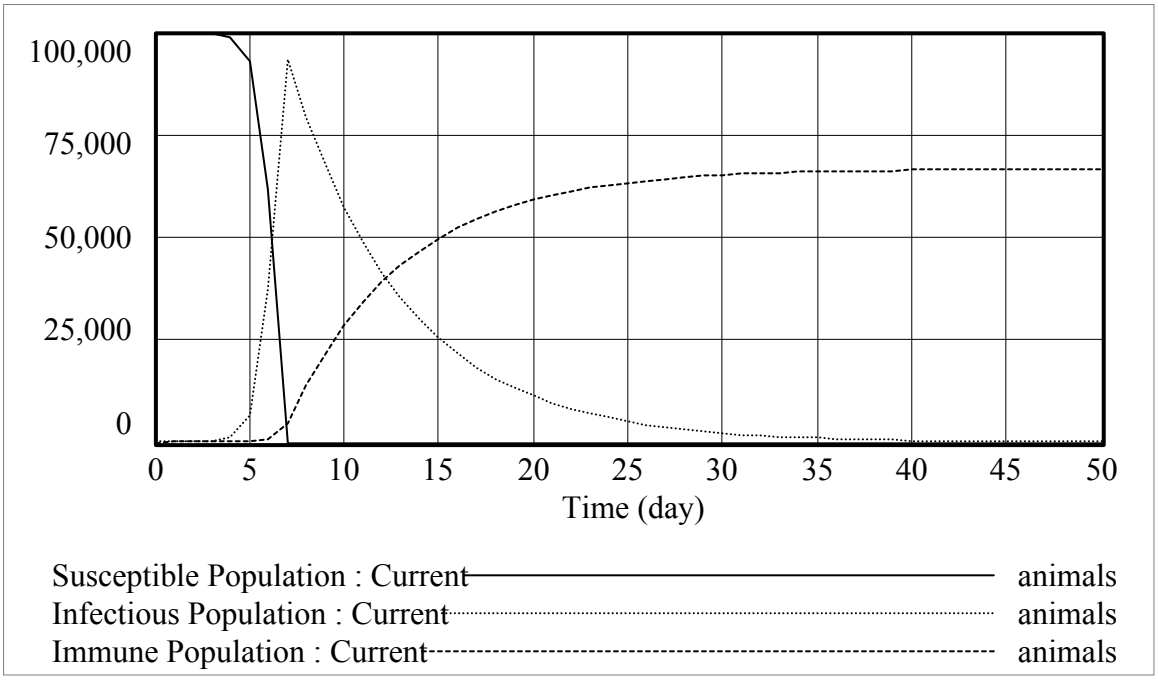
Because we set the units of each equation to animals/day we also need to specify in Vensim that we want the simulation to run on the time of days. (You can also specify hours, months, years, etc.) To do this, select the drop down menu Model>Settings. Select the Time Bounds tab, and next to **Units for Time** click the drop down arrow and select Day. Let's also change the **FINAL TIME** to 50. This will allow Vensim to run for 50 iterations and will then allow for the appropriate scaling of the graph.



Now, click on the little green-shirted running person at the top to run the simulation. If you choose the running person with the little lines, it will give you **slider bars** for each variable so that you can change the values and see what happens.

When the simulation stops, click on the little circle at the top right and when the window pops up, select the graph tab. Click on **New…** to make a quick graph of the results. A new window (see below) will appear that allows you to choose the variables and the options for the graph. After you have created a graph, you can change use the same window to change the appearance by clicking on **Modify** instead of **New**. There are six rows of blanks near the bottom that allow us to select the variables that we'd like to see simultaneously on the same graph. Click on the **Sel** and a list of variables will appear. Pick susceptible population and then ok for the first row. The select infectious population and immune population for the two next rows, respectively. The click the little boxes to the left, under scale, to make all variables have the same scale for the graph. There are many other options here to improve the graph, but this setup will give us a nice start. Click OK to return to the Control Panel and then **display** to see the graph. It should look like the one below.





Through these few quick and simple steps, we've created an epidemic model that captures the crucial aspects of a traditional SIR epidemic model. This can serve as a starting place for crafting much more realistic and useful models. You can immediately test effects of changing parameters or make modifications to customize it for a specific disease or species. To go further, select **Help** and **Vensim Manuals**. All the needed documentation is available electronically and can be accessed as chapters, or by searching. GOOD LUCK AND HAVE FUN!

ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

6. HEALTH ASSESSMENT WORKSHEET FOR ANIMAL MOVEMENTS

Developed By:

AUCKLAND ZOO

Department of Conservation
Te Papa Atawhai

Health Assessment Worksheet for Animal Movements

Introduction

This Worksheet provides a framework for developing quarantine and health screening protocols aimed at minimising disease risks during the movement of wildlife.

This process enables wildlife managers and veterinarians to consider the specific disease risk issues associated with each planned wildlife translocation and to communicate this, via the Worksheet, to all involved in the animal movement. For those people with access to the internet the Worksheet is available as a form on the CBSG website. This form can be completed directly on the computer and then forwarded to others via e-mail.

Situation-Specific Protocols

The Worksheet process provides wildlife managers with the flexibility to develop a protocol consistent with available information, time and resources and specific to the circumstances associated with each individual animal movement.

It is recognised that resources for wildlife management are generally constrained and the data needed to make a quantitative risk assessment is often incomplete. The Worksheet enables managers to work within these constraints and begin the process of identifying gaps in knowledge which can be filled when opportunities arise. **A fundamental underlying principle of this process is that some level of health screening is better than none**.

Diseases of Concern

Identifying which disease or health problems to screen animals for (Section 8 and Appendix 1 of the Worksheet) is the most difficult and potentially time consuming process in developing each protocol. Ideally this should involve a search of all relevant literature and all available unpublished material documenting the disease susceptibilities of the animal species affected by your animal movement. The extent of this search will depend, however, on the time and other resources available to you. You may, initially have to use only locally available records and plan to build on this as you are able. Having compiled your list in Appendix 1 you will then need to evaluate the significance of each health problem to this specific animal movement. This can be done using the process provided in Appendix 1 or you may wish to use one of the other CBSG disease risk assessment processes.

It is the intention of CBSG to collate Worksheet information into a central database. Over time this will provide a valuable information resource on diseases of concern to various animal groups.

Prioritising Health Screening Tests and Disease Control Measures

Table 8 in the Worksheet includes a column in which you are asked to prioritise the health screening tests and disease control measures according to costs, time constraints and animal factors such as body size and stress of handling. This will be a valuable guide to all involved if any one of these factors becomes limiting.

Collecting Baseline Health Data
In planning wildlife translocations give some consideration to the opportunity for collecting baseline health data and, if possible, publishing these. For most non-domestic species there is still very little normal health data available and this makes interpretation of data collected during pre-shipment health screens difficult to evaluate.

Quarantine Duration
There are sometimes good reasons why an animal cannot be quarantined (e.g. time constraints in an emergency wildlife evacuation, excessive stress on the animal when confined etc) or when a less than ideal quarantine duration must be chosen. The Worksheet allows for these contingencies but requires that a reason is given for the duration chosen. This will be valuable to others when planning movements of the same species at a later date or under similar circumstances.

Diagnostic Sample Collection, Storage and Transport.
In recognition of the fact that, in many cases, the persons collecting diagnostic samples from animals in the field will be non-veterinarians, a simple guide to collection, storage and transport of diagnostic samples is provided as an attachment to these notes. It is highly recommended that the techniques are practiced – preferably under veterinary supervision – before samples are collected in the field. The value of the samples will directly reflect the quality of the processes used to collect and handle them.

Appendices
Appendices to the Worksheet include notes on qualitative and quantitative risk analysis methods, diagnostic test specificity and sensitivity and choice of sample size for developing baseline data.

Health Assessment Worksheet Explanatory Notes

1. **Species to be moved**: Enter common and scientific name.
   A separate sheet should be used for each species and each movement.
   A "movement" begins at the original location(s) and ends at the final destination(s) of the animals. Some intermediary locations (e.g. a central quarantine site) may be involved. Quarantine location is noted on page 3 of the Worksheet.

2.a **From**: State current location(s) of animals to be moved (the source(s)).

2.b **To**: State the final location(s) to which the animals will be moved (the destination(s)).

3. **Total number of animals**: Specify total number of animals to be moved.

4. **Animal identification**: Each animal should be individually identified or a group number assigned where individual identification is not possible. (e.g. amphibians or schools of fish).
   ID Number: specify the identification number or code for each individual or group
   ID type: specify the type of identification marker used e.g. Trovan™ or other microchip implant, leg band, ear tag, tattoo, etc. (Note: A means of permanent identification is highly recommended).
   Animal Origin: List each animal's origin as **W** = Wild, **C** = Captive or **U** = Unknown. **B** = Both may be used for groups only.
   ***Age*:** Enter age in years or, where unknown, classify as **Juv** = juvenile or **Ad** = adult
   Sex: List sex as **M** = male, **F** = female or **U** = unknown. **B** = Both may be used for groups only.
   Medical History: If a medical history is available will it accompany this individual or group? Enter **Y** = Yes or **N** = No. If medical history is not available write **N/A**.
   ***Comments*:** Include any pertinent information such as significant disease history, contraceptive implants, neutered etc.

5.a **Movement category**: Check the box that describes this animal movement

5.b **Permits to move animals received**: Circle Yes or No as appropriate. In the box below, list all permits received and their expiration dates.

6.a **Project manager**: Enter the name of the person responsible for coordinating this animal movement and his/her telephone/fax numbers and email address.

6.b **Title, Institution**: Enter the Project Manager's position in the organisation and the name of the organisation

7. **Project veterinarian** – Enter the name of the designated veterinary advisor to this project and his/her telephone/fax numbers and email address.

8**. Health screen and control measures for diseases of concern:**

To identify the diseases of concern for this movement, complete the table in Appendix 1 (see explanatory notes for this below). Available information, time and resources will determine the level of detail to be used in identifying potential diseases of concern.

Disease/Health Problem: List the diseases or health problems to be specifically addressed for this animal movement.

Recommended diagnostic method or control measures: Indicate the diagnostic method(s) (e.g. specific serological test, physical exam etc) or control measures (e.g. vaccination, control of parasite vectors etc) to be used to screen for, or prevent exposure to, the disease or health problem of concern.

Priority: Rank each disease/health problem according to its importance for this animal movement (where **1** = highest priority and **5** = lowest priority).

9. **Diagnostic methods and sample collection**:

For guidance on sample collection and handling refer to the attached: *"The collection, storage and transport of diagnostic samples from birds and reptiles"* or other suitable texts.

Sample**:** For each diagnostic method listed in table 8 above, list the type of sample to be collected (be specific eg. "Whole blood" or "serum" or "plasma" – not just 'blood')

Test: Enter the specific diagnostic test the sample is to be used for.

Minimum sample amount: Enter the minimum quantity (i.e. volume or weight) of sample to be collected for the specified test (check with laboratory if unsure).

Sample collection date: Enter the date the sample is to be collected. To minimise handling of animals this will usually be on the day of capture and/or transfer into quarantine.

Sample to be forwarded to: Insert the name of the testing facility and a contact name/address/telephone number. (Note: It is recommended that you contact your sample testing facility in advance to advise the date of sample collection and to ask for guidance on sample handling, storage and shipment. Also check any permit requirements for collection and shipment of samples).

10 **Prophylactic Treatments, Vaccinations and Control Measures**: List all treatments (including drug names, doses and route of administration) and vaccinations (including type of vaccine) to be given before animals are exposed to destination populations. All other disease control measures, such as disinfection of crates for transportation etc., should also be noted.

11. **Location of quarantine**: Geographic location of quarantine (e.g. zoo, park, specific island)

12. **Facility**: Specific building or site of quarantine.

13. **Quarantine duration**: Insert the dates the quarantine period begins and ends and total number of days the animals will be in quarantine. Provide an explanation for the duration of quarantine chosen or why quarantine is not to be used, if this is the case. This decision should consider disease factors, test requirements, and animal husbandry issues (e.g. stress in captivity). If no quarantine go to 16.

14. **Person supervising quarantine**: Insert the name of the person responsible for maintaining animal health and quarantine status. Include his/her contact details.

15. **Quarantine equipment and set-up**: Check appropriate boxes for the items to be organised.

16. **Budget**: Identify all costs associated with this animal movement project. This could include costs for personnel, equipment, animal feed, laboratory services, shipping of samples, veterinary fees and expenses etc. Where appropriate indicate which budget to debit these expenses to. If no specific budget for this movement go to 17.

17.a **Results of health screen**: For each animal itemise any <u>positive or abnormal</u> diagnostic test results. The project veterinarian should comment on the significance of these results in the far right column. Where no significant results were found write NSF (No Significant Findings).

17.b **Overall assessment and comment on results**: Veterinarian to comment on overall results including level of confidence in diagnostic tests and examinations.

18 **Movement recommendations**: Following review and discussion of the quarantine and health screening results check the appropriate box.

19 **Explanation and justification of animal movement recommendation**: Justify the recommendation and include the methods (qualitative and/or quantitative) used to evaluate the disease risks – see other CBSG disease risk assessment tools.

20 **Follow up actions**: Itemise all actions to be taken to follow up this movement e.g. post-transfer monitoring, health surveillance of in-contact populations at destination sites, review of protocol including collection of baseline data to improve risk assessment data for future movements etc.

21 **Signed off by**: At the completion of the project the form should be signed off and dated by the Project Manager and Veterinarian.

Appendix 1: Infectious and Non-Infectious Disease Susceptibilities of Species Affected by this Animal Movement

This table is provided to help compile as comprehensive a list of diseases as possible to which this and other relevant species are susceptible. Veterinary assistance in developing this list is strongly recommended.

A box is provided below the table to list the species **at source and destination sites** most likely to be affected by diseases that may be transmitted as a result of this animal movement. This includes in-contact people, wildlife and domestic animals.

Explanation of table columns:

Disease/Problem: Insert specific diseases or health problems (including genetic, environmental) to which these species are susceptible.

Using available knowledge and sources of information each disease should be ranked High (H), Medium (M) or (Low) against the characteristics in the next five columns. On this basis, a qualitative assessment of the relative risk associated with each disease can be made by assigning

it an overall High, Medium or Low ranking. A quantitative analysis using more sophisticated methods may be appropriate in some cases – see other CBSG disease risk assessment tools.

Exposure threat: the likelihood that an animal or population will be exposed and adversely affected by a pathogenic agent e.g. a microorganism, toxic agent, deleterious gene etc.

Infectivity: The characteristic of a microorganism that allows it to infect and subsequently survive and multiply within a susceptible host.

Pathogenicity: The host-specific ability of an agent to cause disease or otherwise induce pathological changes in a susceptible host

Transmissibility: The capacity for a disease agent to be transferred directly or indirectly from one susceptible host to another.

Susceptibility: The state of being readily affected by a pathogen; a lack of resistance to a pathogen.

Disease of Concern? Y/N: Insert **Y** (= yes) for any disease or health problem that receives an overall ranking of Medium or High. These are the diseases of concern to be listed in Section 8 of the Worksheet.

Source(s) of information: Number each source of information and reference this to a bibliography which should be attached to the Worksheet.

Health Assessment Worksheet for Animal Movements

(Please refer to explanatory notes while completing this Worksheet)

1. SPECIES TO BE MOVED: _____

2a. FROM: _____ **2b. TO:** _____

3. TOTAL NUMBER OF ANIMALS: _____

4. ANIMAL IDENTIFICATION: (attach additional sheets if needed)

5a. MOVEMENT CATEGORY: Wild to wild ☐ Wild to captivity ☐ Captivity to wild ☐ Captivity to captivity ☐

5b. PERMITS TO MOVE ANIMALS RECEIVED **YES** **NO** (Circle one)

(List all permits and their expiry dates)

_____

_____

_____

_____

_____

6a. PROJECT MANAGER: _____ Tel. _____ Fax _____

6b. TITLE, INSTITUTION: _____ E-mail: _____

7. PROJECT VETERINARIAN: _____ Tel. _____ Fax. _____ E-mail: _____

8. HEALTH SCREEN & CONTROL MEASURES FOR DISEASES OF CONCERN TO THIS ANIMAL MOVEMENT

(Diseases of Concern are as identified from Appendix 1)

9. DIAGNOSTIC METHODS AND SAMPLE COLLECTION

For results refer to Section 17a on this form

*Contact your sample testing facility to advise of date of sample collection and obtain guidance on sample handling, storage and shipment. Also check permit requirements for collection and shipment of samples.

10. PROPHYLACTIC TREATMENTS, VACCINATIONS AND CONTROL MEASURES

For documentation refer to individual animal records.

Quarantine Details *If no quarantine explain reason(s) in 13 below and go to 16.*

11. LOCATION OF QUARANTINE: _____

12. FACILITY: _____

13. QUARANTINE DURATION (Based on animal management, diagnostic and disease criteria):

Begins *(date)* _____ **Ends** *(date)* _____ **Total days:** _____

Specify reason(s) for the duration below:

_____

_____

_____

14. PERSON SUPERVISING QUARANTINE: _____

Tel. _____ Fax. _____ E-mail _____

15. QUARANTINE EQUIPMENT AND SET UP:

Check

- ☐ **"Quarantine - No Unauthorized Entry Sign"**
- ☐ **Insect/rodent traps/screens/baits**
- ☐ **Diagnostic sample collection, storage and transport equipment**
- ☐ **Lock for facility**
- ☐ **Footbath/boot changes**
- ☐ **Protective clothing**
- ☐ **Cage furniture as appropriate for species**
- ☐ **Animal record forms, pens**

Check

- ☐ **Bags for waste disposal**
- ☐ **Feeding, watering and cleaning utensils**
- ☐ **Animal capture and restraint equipment**
- ☐ **Quarantine register**
- ☐ **Animal caregiver personal health check**
- ☐ **Written quarantine protocol**
- ☐ **Briefing of veterinarian with quarantine supervisor**
- ☐ **Other:** _____

16. BUDGET: (I*f not required go to 17*)

17a. RESULTS OF HEALTH SCREEN

17b. OVERALL ASSESSMENT AND COMMENTS ON RESULTS

18. Movement Recommendation (Check appropriate box)

*If move delayed state time and condition for release:

19. EXPLANATION AND JUSTIFICATION FOR ANIMAL MOVEMENT RECOMMENDATION:

20. FOLLOW UP ACTIONS

21. SIGNED OFF BY: _____ **DATE:** _____

Project Manager

_____ **DATE:** _____

Project Veterinarian

APPENDIX 1: INFECTIOUS AND NON-INFECTIOUS DISEASE SUSCEPTIBILITIES OF SPECIES AFFECTED BY THIS ANIMAL MOVEMENT*

(List below potential in-contact wildlife, domestic animals and humans. Veterinary assistance is strongly recommended for developing this list)

Disease/Problem	Exposure threat	Infectivit	Pathogenicit	Transmissibilit	Susceptibili	Disease of Concern? Y/N	Source(s) of information

*For each disease rank High (H), Medium (M) or Low (L) for infectivity, pathogenicity, transmission and susceptibility. Select as "disease of concern" if, overall` the veterinarian ranks the disease or health problem as a Moderate to High risk based on this, or a more quantitative, analysis.

** Number each source of information and reference this to a bibliography which should be attached to this document

Species potentially affected by this movement:

_____

_____

_____

_____

Disease Risk Assessment for Animal Movements Database

(created by John S. Williams, CBSG)

The Risk Application is used for entering data into the Health Assessment Worksheet for Animal Movement. This database is available electronically on the enclosed CD.

The standard version of the program, version 1.5 or 1.6 works with Microsoft ACCESS, part of Microsoft Office 2000 Professional, or with Microsoft Office XP Professional. A second version of the program that will work with computers that have Microsoft Access 97 installed. It is functionally equivalent to the Office 2000 version, and places the data in the identical format.

In addition, there is a runtime version that will work on most computers that do not have any version of Microsoft ACCESS installed.

TO INSTALL THE ACCESS 2000 VERSION:

SYSTEM REQUIREMENTS: Windows 98, (or higher, such as Windows ME, Windows 2000, Windows XP) and 64 MB of RAM.

The application comes with two files:
RISK 1-6.MDB – The Program File
RISK_BE.MDB – The Data File.

These two files should be copied into the following location:
C:\DATA\RISK

These two files are small enough to fit on a floppy diskette. If you have the zipped version, then you may unzip them to the directory C:\DATA\RISK.

To start the program, open up Microsoft Access, then open the file RISK 1-6.MDB. (The slightly older version RISK 1-5.MDB has the same data format).

TO INSTALL THE ACCESS 97 VERSION

SYSTEM REQUIREMENTS: Windows 98 or higher, 32 MB of RAM.
To install the ACCESS 97 version, run the program RISK97.EXE, which will install the ACCESS 97 version of the program onto the computer. It will install two files:
RISK1-5.97.MDB – the Program file
RISK_BD.MDB – The Data File

To start the program, open up Microsoft Access 97, and then open the file RISK1-5.97.MDB.

TO INSTALL THE RUNTIME VERSION

SYSTEM REQUIREMENTS: Windows 98 or higher, 32 MB of RAM, CD-ROM.

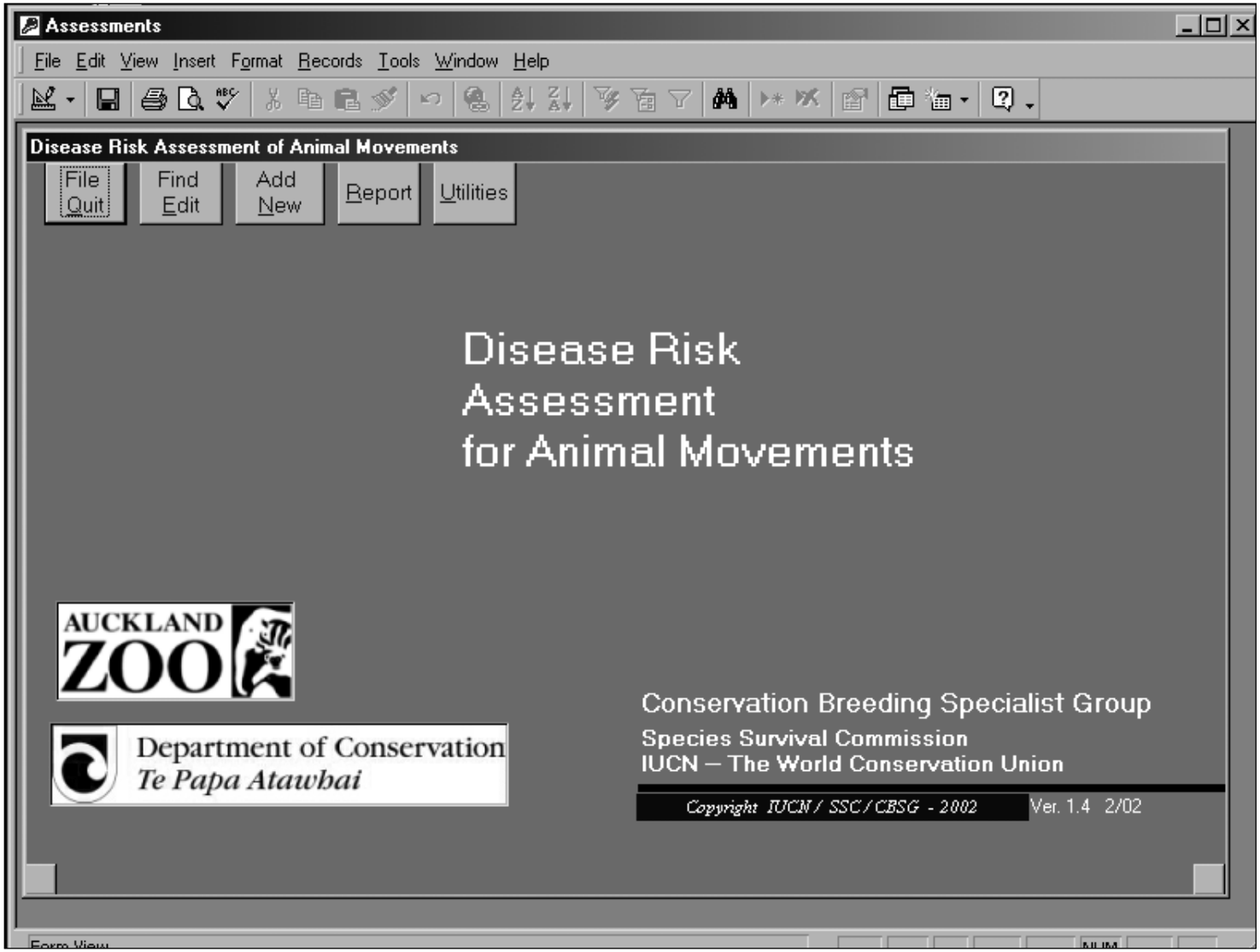
Note: This version will require about 15 MB of memory on your HARD DISK. It installs a limited runtime version of Microsoft Access in addition to the program.

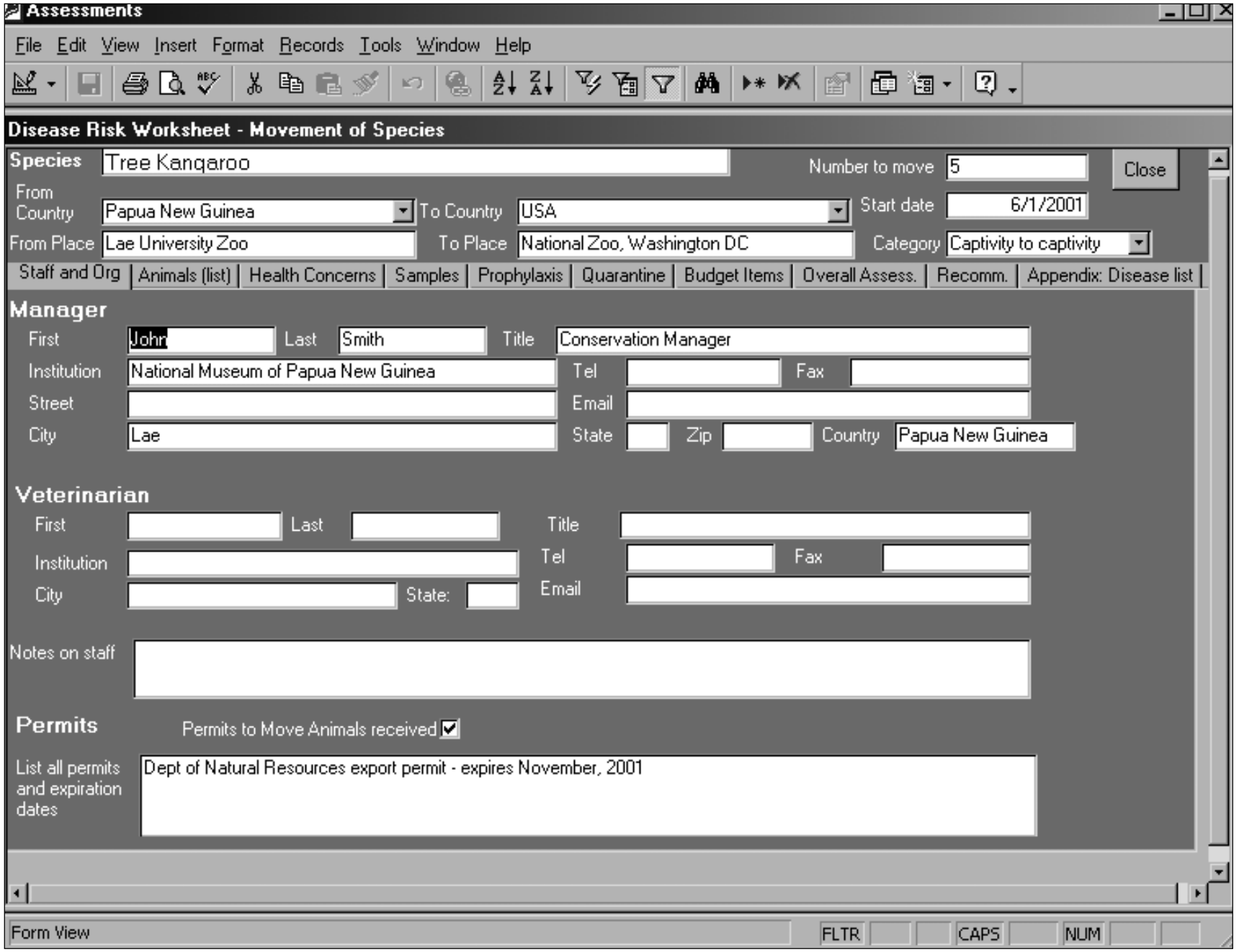
1. Place the CD-ROM containing the RISK runtime version.
2. Click on the Start button (lower left hand corner of the Windows Desktop Screen), click on "RUN", then browse for the program "STARTUP.EXE, which may be found on the CD in the "RiskRunTime" directory.
3. After you have found the program, click on OK, to install the program.
4. Follow all the defaults, the program must be installed in the suggested directory.

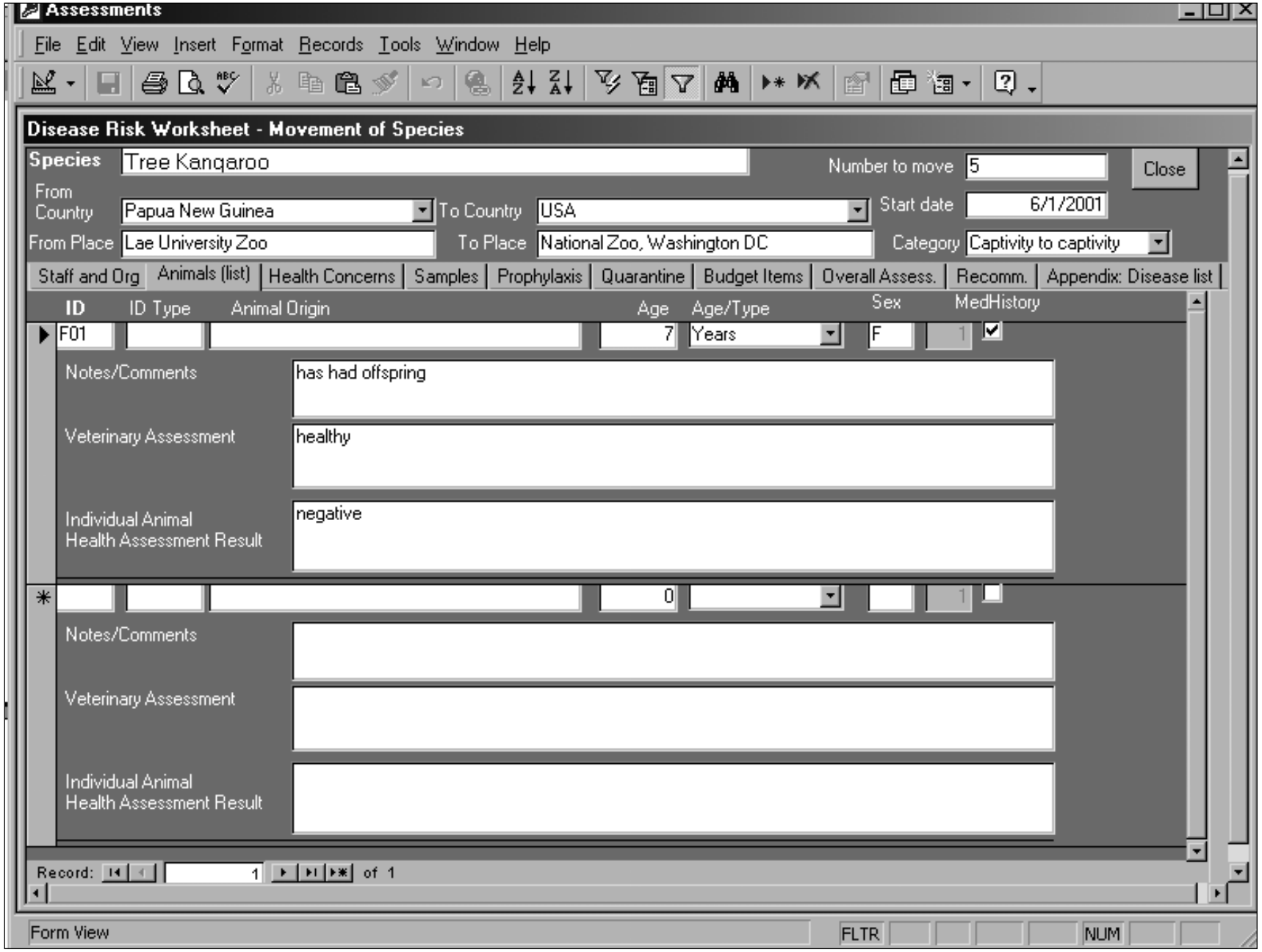
After the installation has been completed, you will be required to "reboot" or shut down and restart your computer. Upon start up, you will find the RISK program in the program list. (Click on the Start Button, then on "Programs", and you should find RISK, probably at the bottom of the list.

Start the program, and proceed to enter data.

A sample data entry for Tree Kangaroos:




Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
Close
From Country
Papua New Guinea
To Country
USA
Start date
6/1/2001
From Place
Lae University Zoo
To Place
National Zoo, Washington DC
Category
Captivity to captivity
Staff and Org
Animals (list)
Health Concerns
Samples
Prophylaxis
Quarantine
Budget Items
Overall Assess.
Recomm.
Appendix: Disease list
Manager
First
John
Last
Smith
Title
Conservation Manager
Institution
National Museum of Papua New Guinea
Tel
Fax
Street
Email
City
Lae
State
Zip
Country
Papua New Guinea
Veterinarian
First
Last
Title
Institution
Tel
Fax
City
State:
Email
Notes on staff
Permits
Permits to Move Animals received
List all permits and expiration dates
Dept of Natural Resources export permit - expires November, 2001
Form View
FLTR
CAPS
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
Close
From Country
Papua New Guinea
To Country
USA
Start date
6/1/2001
From Place
Lae University Zoo
To Place
National Zoo, Washington DC
Category
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Staff and Org
Animals (list)
Health Concerns
Samples
Prophylaxis
Quarantine
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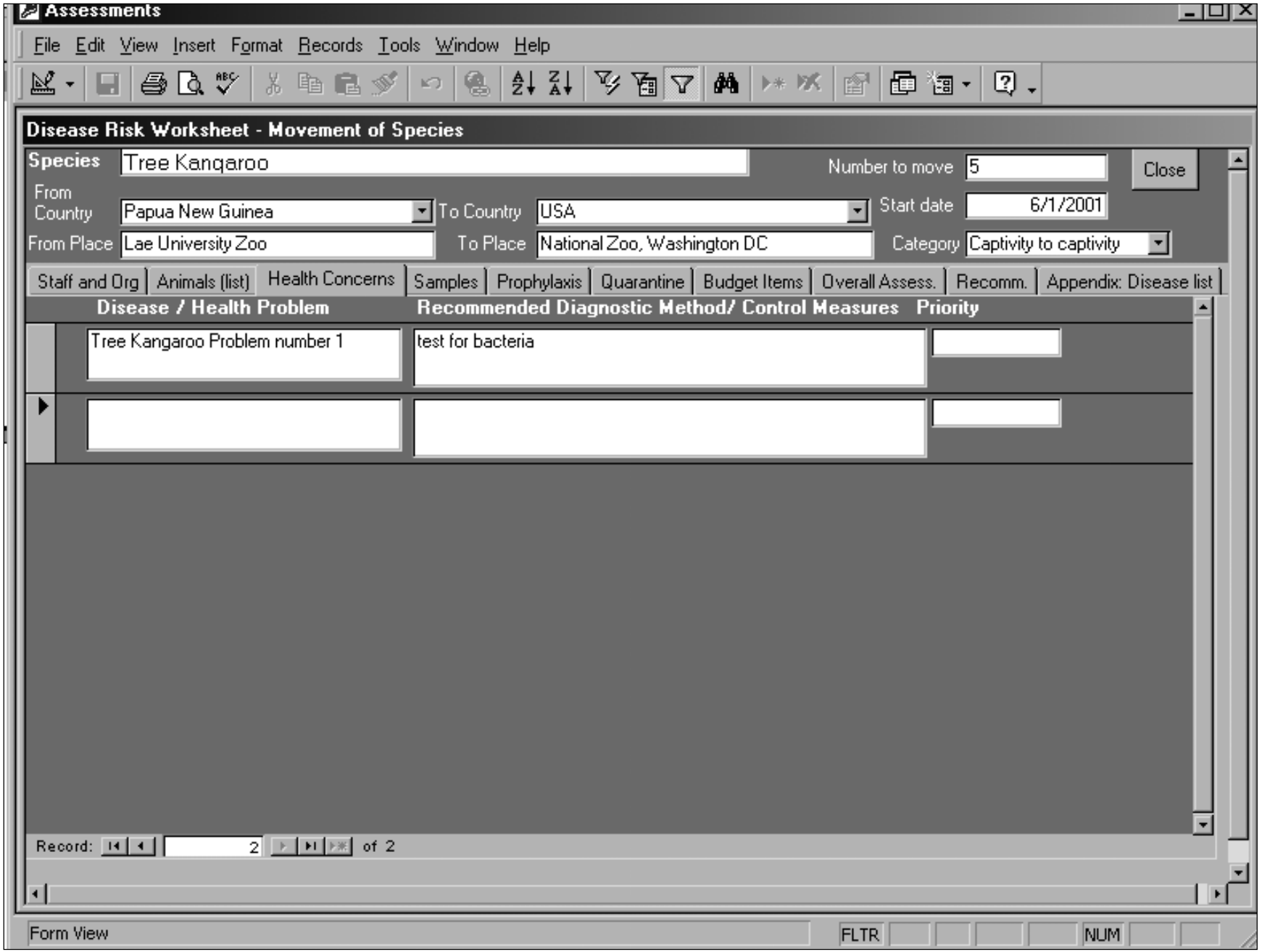
Overall Assess.
Recomm.
Appendix: Disease list
ID
ID Type
Animal Origin
Age
Age/Type
Sex
MedHistory
F01
7
Years
F
1
Notes/Comments
has had offspring
Veterinary Assessment
healthy
Individual Animal Health Assessment Result
negative
0
Notes/Comments
Veterinary Assessment
Individual Animal Health Assessment Result
Record:
1
of 1
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
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5
Close
From Country
Papua New Guinea
To Country
USA
Start date
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To Place
National Zoo, Washington DC
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Quarantine
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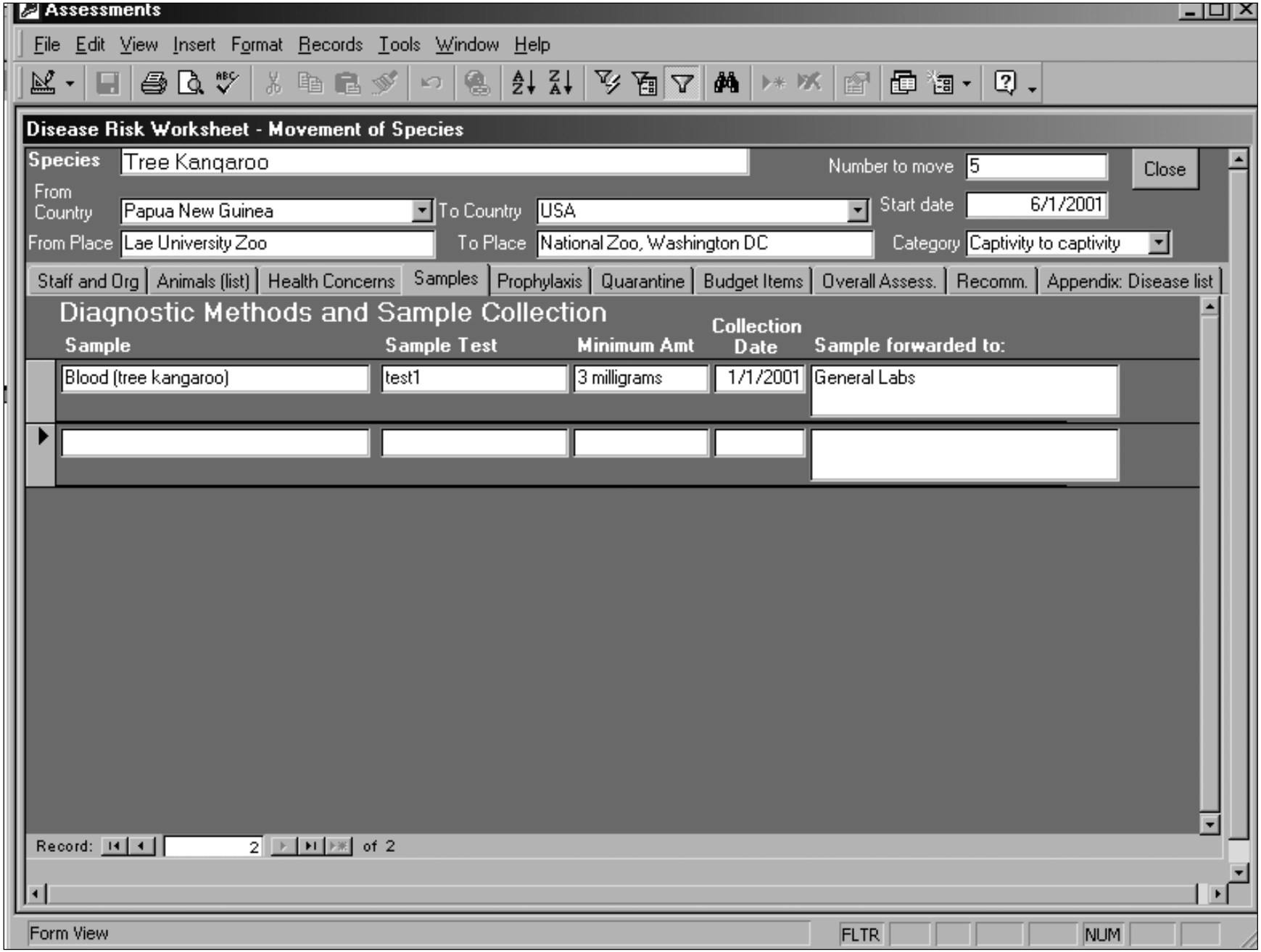
Overall Assess.
Recomm.
Appendix: Disease list
Disease / Health Problem
Recommended Diagnostic Method/ Control Measures
Priority
Tree Kangaroo Problem number 1
test for bacteria
Record:
2
of 2
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species Tree Kangaroo
Number to move 5
Close
From Country Papua New Guinea
To Country USA
Start date 6/1/2001
From Place Lae University Zoo
To Place National Zoo, Washington DC
Category Captivity to captivity
Staff and Org
Animals (list)
Health Concerns
Samples
Prophylaxis
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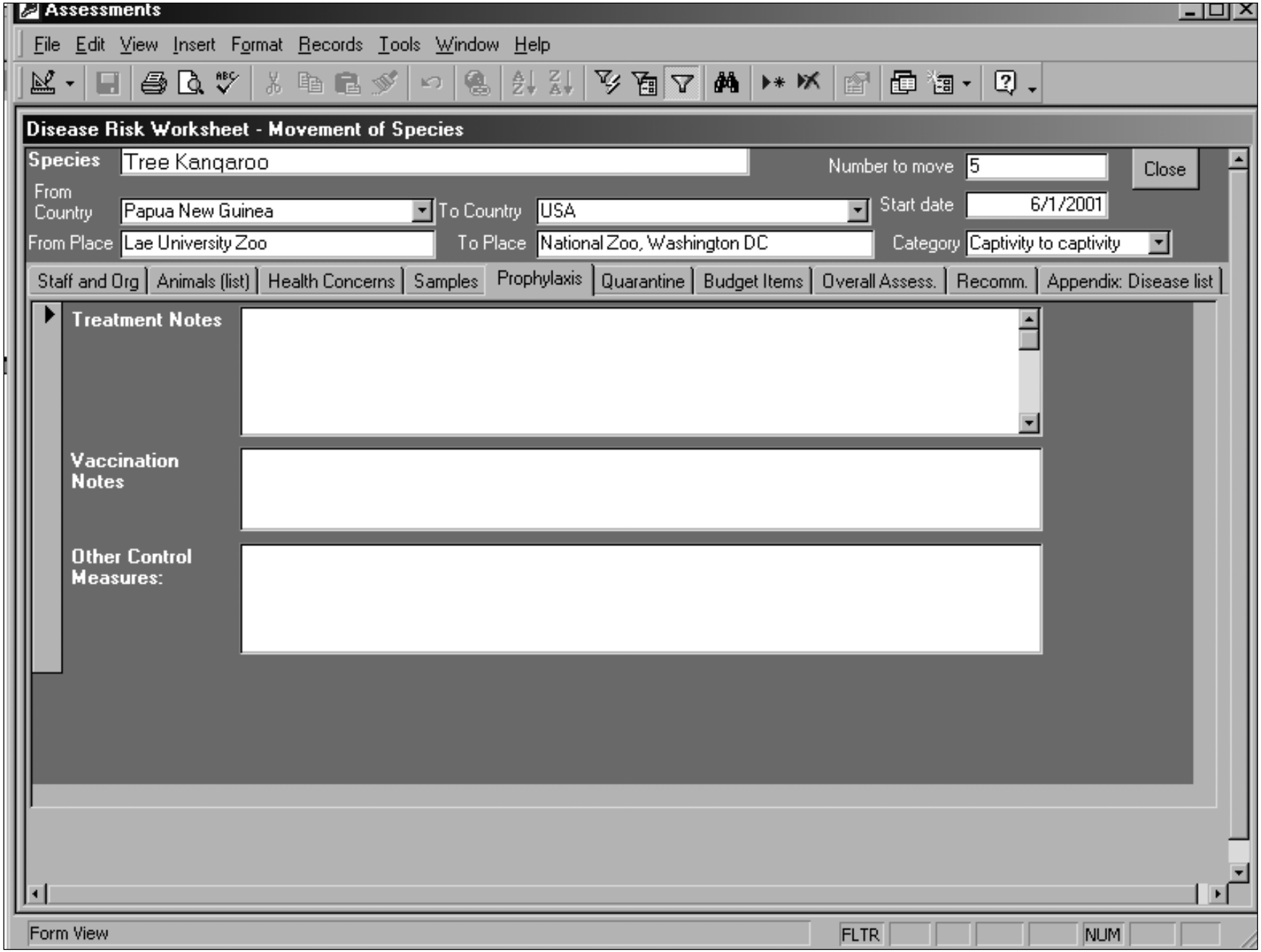
Recomm.
Appendix: Disease list
Diagnostic Methods and Sample Collection
Sample
Sample Test
Minimum Amt
Collection Date
Sample forwarded to:
Blood (tree kangaroo)
test1
3 milligrams
1/1/2001
General Labs
Record: 2 of 2
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
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From Country
Papua New Guinea
To Country
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Quarantine
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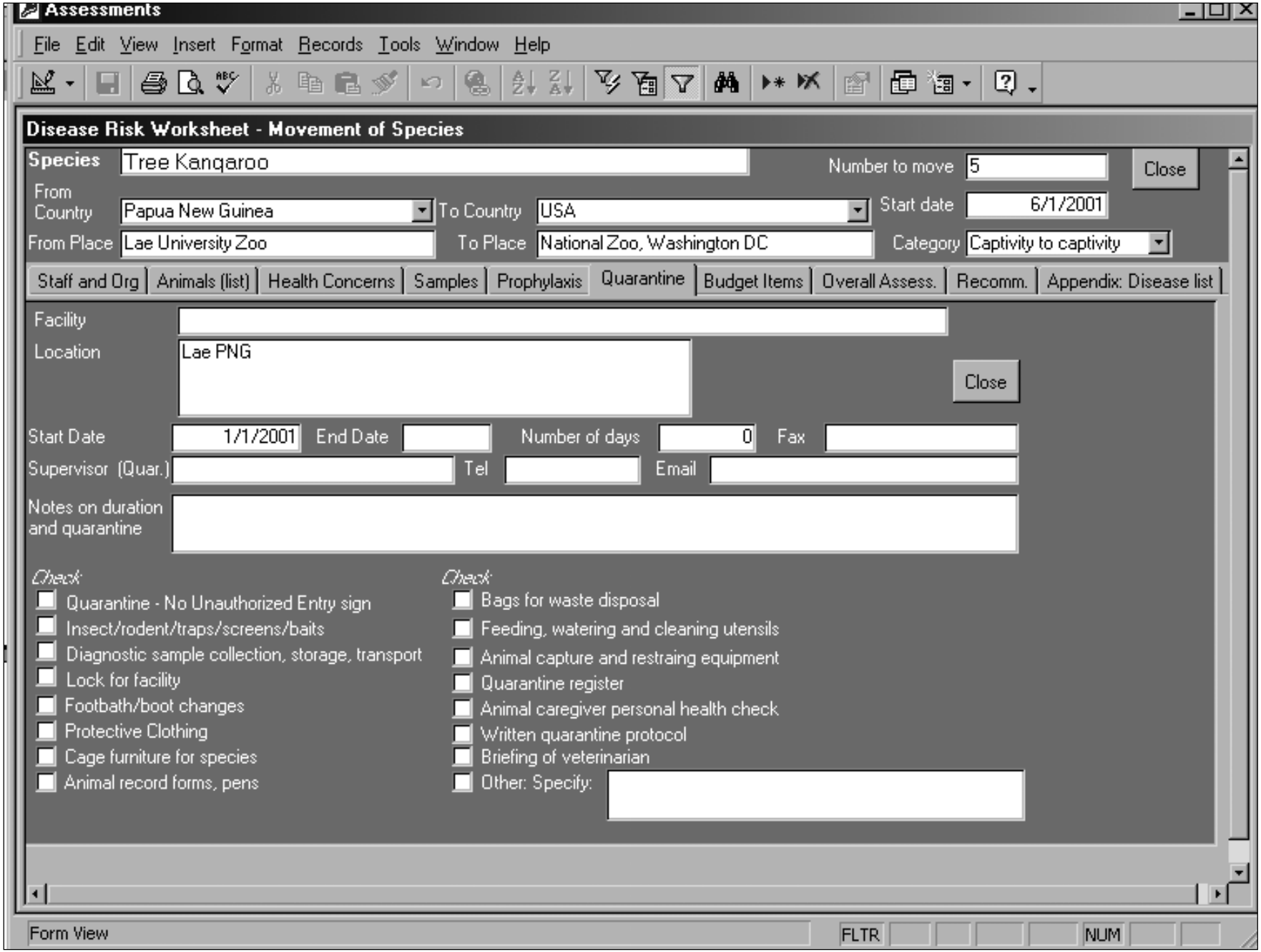
Overall Assess.
Recomm.
Appendix: Disease list
Treatment Notes
Vaccination Notes
Other Control Measures:
Form View
FLTR
NUM


Assessments
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Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
Close
From Country
Papua New Guinea
To Country
USA
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Lae University Zoo
To Place
National Zoo, Washington DC
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Samples
Prophylaxis
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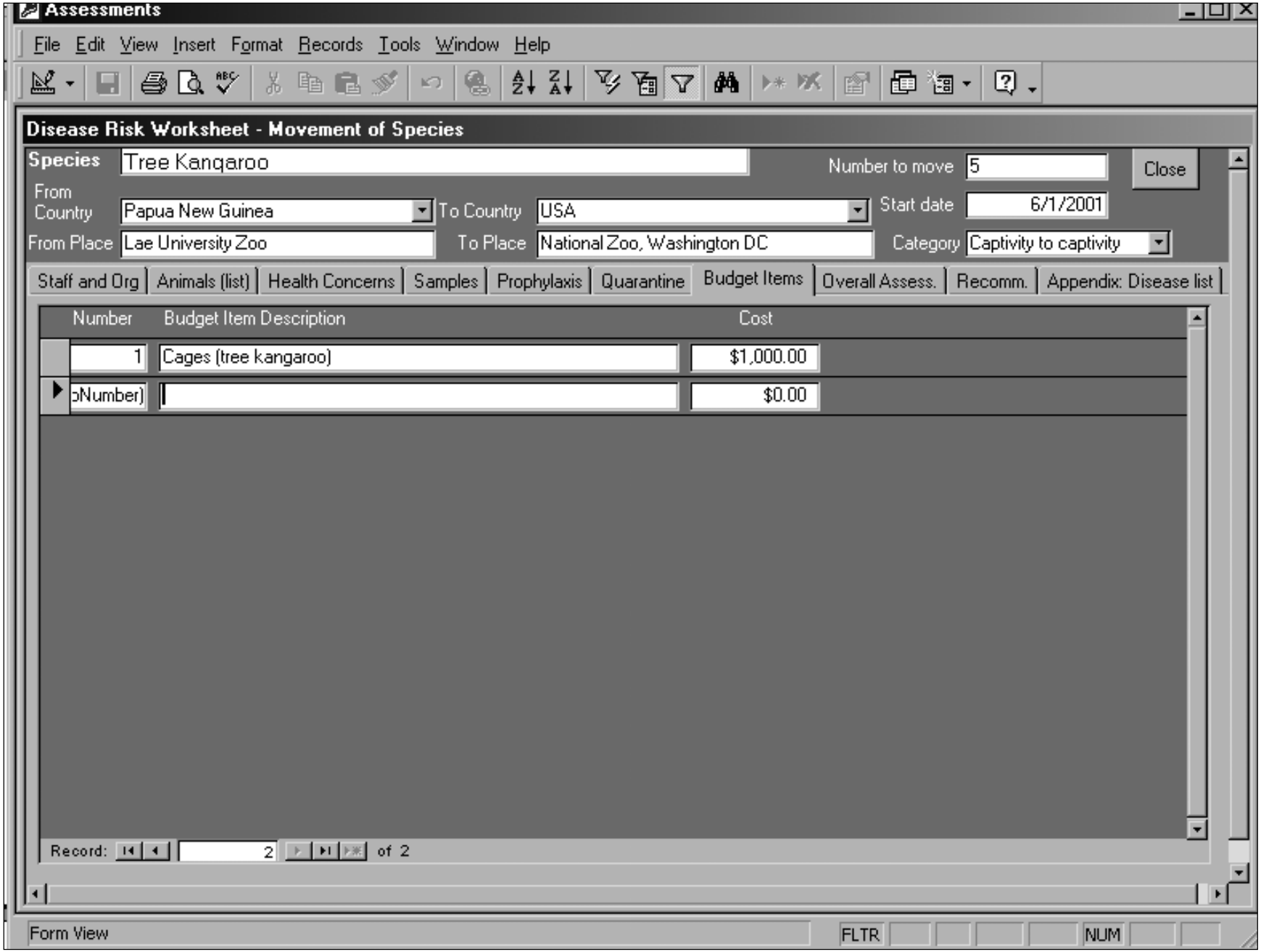
Budget Items
Overall Assess.
Recomm.
Appendix: Disease list
Facility
Location
Lae PNG
Close
Start Date
1/1/2001
End Date
Number of days
0
Fax
Supervisor (Quar.)
Tel
Email
Notes on duration and quarantine
Check
Quarantine - No Unauthorized Entry sign
Insect/rodent/traps/screens/baits
Diagnostic sample collection, storage, transport
Lock for facility
Footbath/boot changes
Protective Clothing
Cage furniture for species
Animal record forms, pens
Check
Bags for waste disposal
Feeding, watering and cleaning utensils
Animal capture and restraing equipment
Quarantine register
Animal caregiver personal health check
Written quarantine protocol
Briefing of veterinarian
Other: Specify:
Form View
FLTR
NUM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File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
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Quarantine
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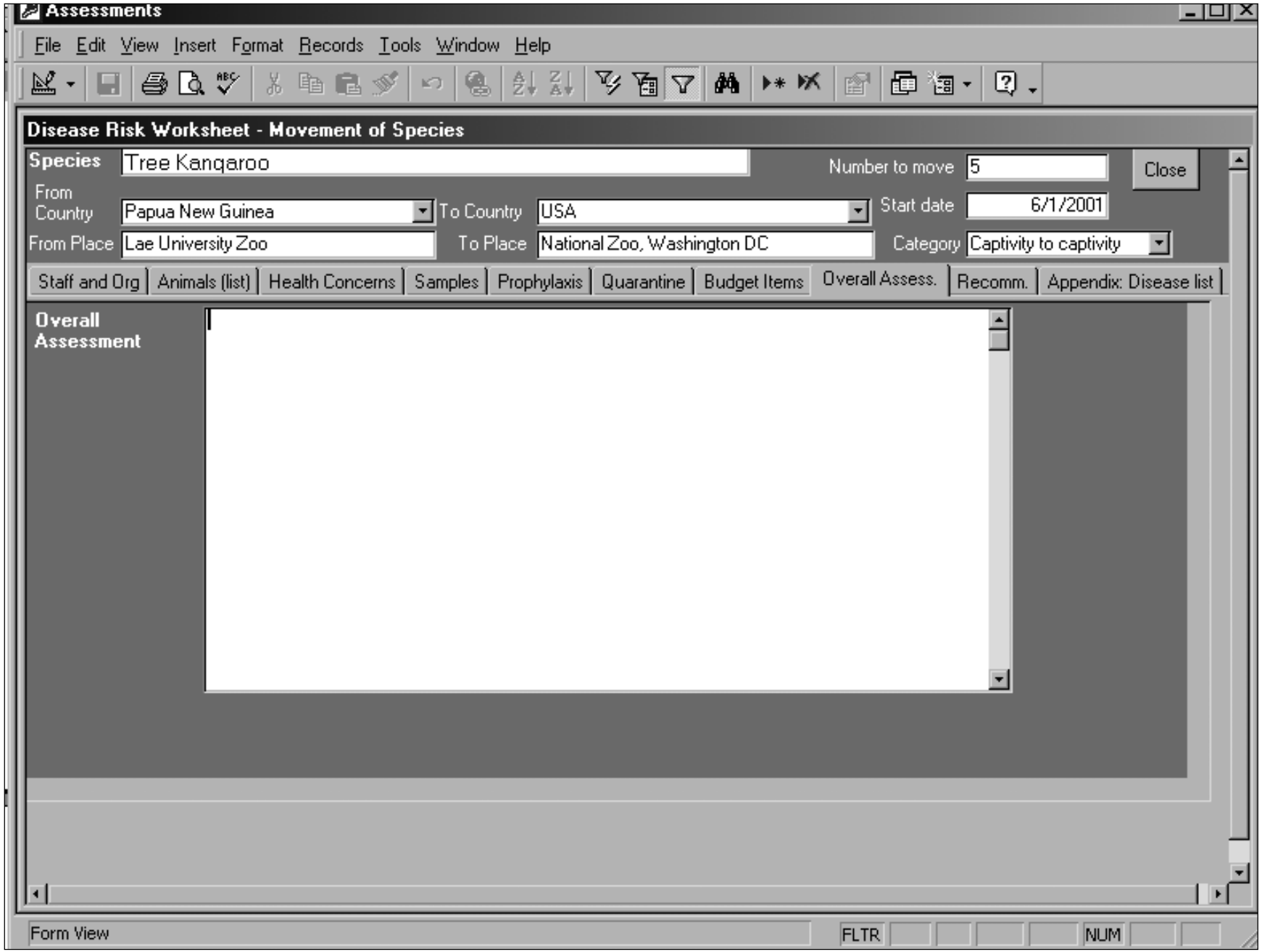
Overall Assess.
Recomm.
Appendix: Disease list
Number
Budget Item Description
Cost
1
Cages (tree kangaroo)
$1,000.00
bNumber)
$0.00
Record:
2
of 2
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
Number to move
5
Close
From Country
Papua New Guinea
To Country
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Lae University Zoo
To Place
National Zoo, Washington DC
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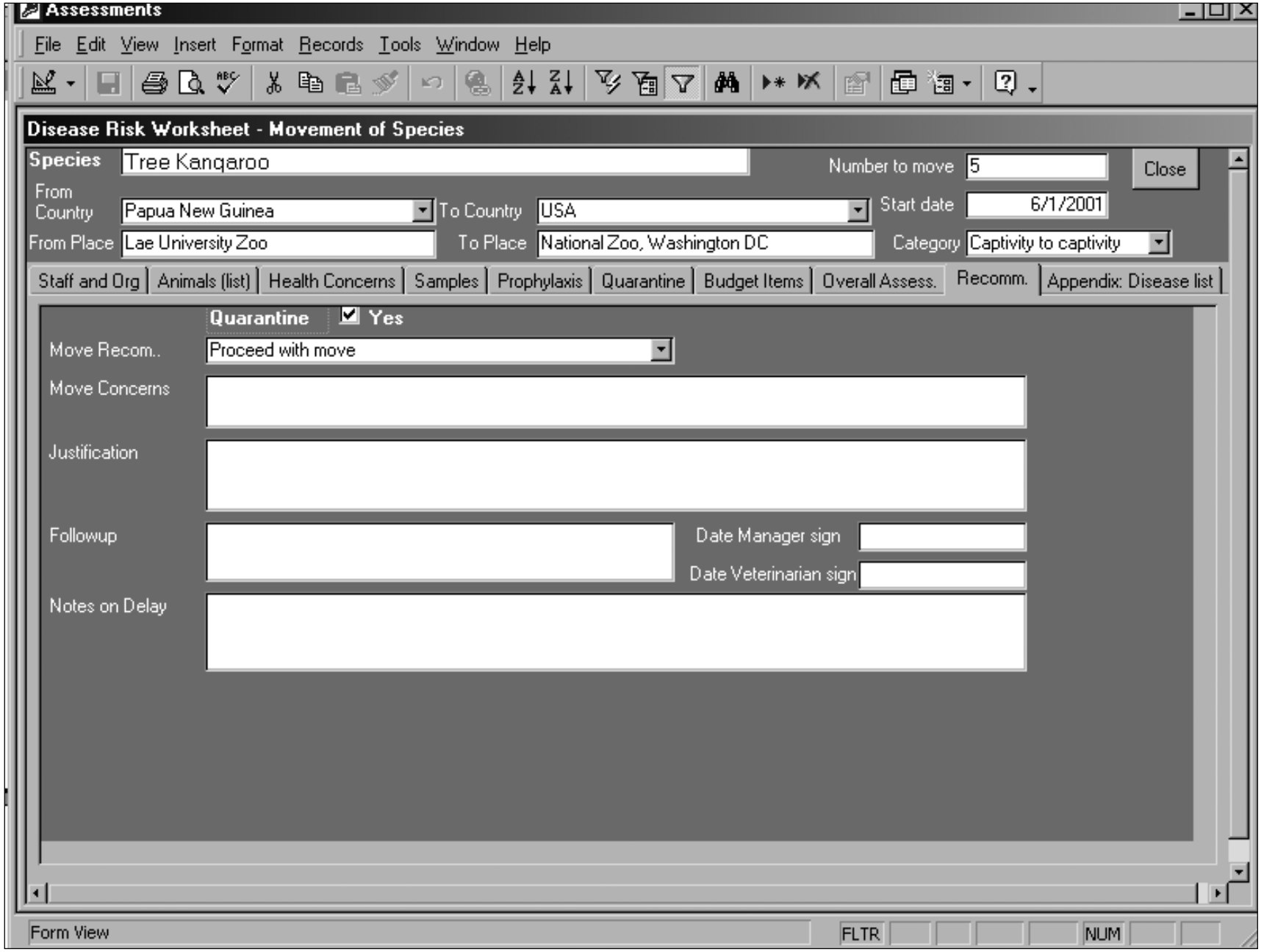
Health Concerns
Samples
Prophylaxis
Quarantine
Budget Items
Overall Assess.
Recomm.
Appendix: Disease list
Overall Assessment
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species
Tree Kangaroo
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From Country
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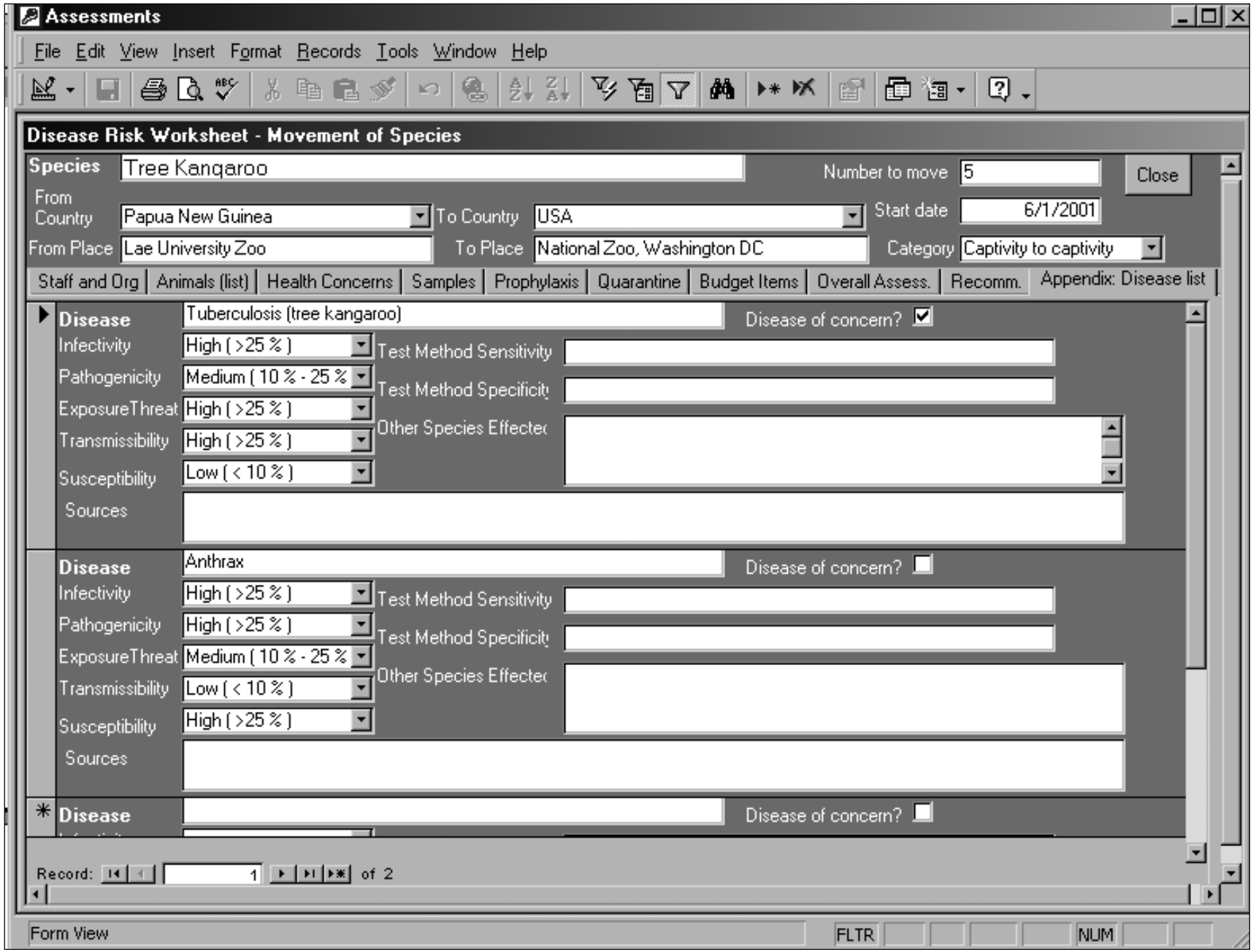
Recomm.
Appendix: Disease list
Quarantine
Yes
Move Recom..
Proceed with move
Move Concerns
Justification
Followup
Date Manager sign
Date Veterinarian sign
Notes on Delay
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Disease Risk Worksheet - Movement of Species
Species Tree Kangaroo
Number to move 5
Close
From Country Papua New Guinea
To Country USA
Start date 6/1/2001
From Place Lae University Zoo
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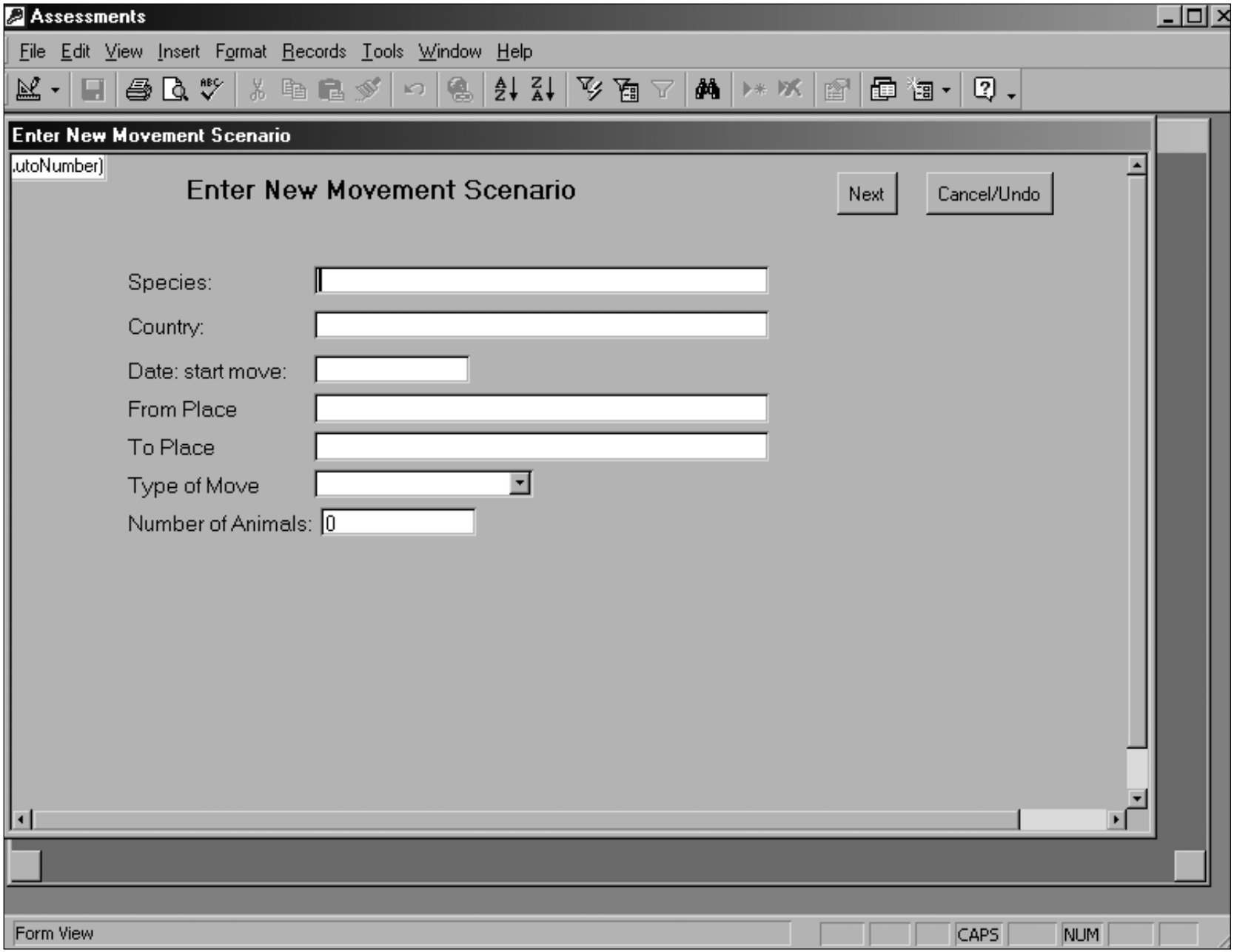
Category Captivity to captivity
Staff and Org | Animals (list) | Health Concerns | Samples | Prophylaxis | Quarantine | Budget Items | Overall Assess. | Recomm. | Appendix: Disease list
Disease Tuberculosis (tree kangaroo)
Disease of concern?
Infectivity High (>25 %)
Pathogenicity Medium (10 % - 25 %
ExposureThreat High (>25 %)
Transmissibility High (>25 %)
Susceptibility Low (< 10 %)
Test Method Sensitivity
Test Method Specificity
Other Species Effected
Sources
Disease Anthrax
Disease of concern?
Infectivity High (>25 %)
Pathogenicity High (>25 %)
ExposureThreat Medium (10 % - 25 %
Transmissibility Low (< 10 %)
Susceptibility High (>25 %)
Test Method Sensitivity
Test Method Specificity
Other Species Effected
Sources
Disease
Disease of concern?
Record: 1 of 2
Form View
FLTR
NUM


Assessments
File Edit View Insert Format Records Tools Window Help
Enter New Movement Scenario
utoNumber)
Enter New Movement Scenario
Next
Cancel/Undo
Species:
Country:
Date: start move:
From Place
To Place
Type of Move
Number of Animals:
0
Form View
CAPS
NUM

ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

7. HEALTH ASSESSMENT WORKSHEET MANUAL

Test Considerations Appendix

Appendix for the Quarantine Health Sheet

Considerations when testing small populations for disease

Disease can play an important role in regulating the distribution and abundance of wildlife populations. The effects of avian malaria on Hawaiian forest birds and canine distemper on black-footed ferrets are two examples that demonstrate the role that disease can play in pushing populations toward extinction. The presence or absence of disease in a population is an important consideration when determining the risks associated with animal movements. Diagnostic testing is needed to determine the disease status of animals in a population. The test results are crucial and are needed by managers and veterinarians to make appropriate decisions regarding animal movements and to provide effective health care for individuals in the population. In order to accurately assess the risks one should strive to obtain results that most accurately reflect the disease status of the population. There are several factors that influence the outcome of the results: the number of animals being tested (the sample size), the composition of the sample (for example the age distribution of the individuals in the sample population) and the quality of the tests being used. Good sampling technique requires that 1) the animals selected for testing are representative of the population as a whole and that 2) an appropriate number of animals are tested to ensure that if the disease is present it will be detected. These techniques assume that a large population is available from which one can select a certain number of animals to sample. It also assumes that one has the ability to randomly select the composition of the sample. Chronic low levels of disease can be present in populations but may go undetected if sampling methods are not appropriate. When dealing with captive and translocation populations these assumptions rarely hold true as the captive or translocation population is usually the sample population.

Sample size and composition

Even if we define our sample population as the entire captive or translocation population, the sample can not be considered random and the sample size will be less than optimal. These limitations do not diminish the importance of or the need for diagnostic testing rather these factors simply need to be considered when interpreting test results and assessing the risks associated with animal movements.

Does the composition of the sample population bias the test results in anyway? Are there peculiarities in the sample population such as the sex ratio, age distribution or genetic makeup of the sample that could affect the outcome of the results? For example if the diagnostic test tests for antibody titers to a specific disease but sufficient titers generally do not develop until an animal is 2 or 3 years of age a sample population comprised of animals that are 1-4 years of age may bias your results. If all individuals in the population test negative, one must consider whether there is still a probability that some animals in the population may be harboring disease.

Sample size is important because it influences the probability of detecting disease if disease is present. While we may lack the ability to change the sample size, understanding the importance of sample size is important because it influences the outcome of the test results. When done correctly sampling can yield an accurate estimate of disease status of the population (i.e. is the disease present in the population and if it is what percentage of the animals is affected?).

Quality of the diagnostic test

Another important factor when testing for disease is the quality of the diagnostic test. The quality of a test is determined by its ability to distinguish between those animals that are diseased and those that are not. The quality of the diagnostic test should be considered when selecting the test (or tests) to be performed and the results must be interpreted in light of the quality of the test. The sensitivity of a test is defined as the ability of a test to identify correctly those individuals who have the disease (animals who are true positives). For example, in a flock of 150 birds 100 birds have disease X, if we test the flock and 80 of the infected birds are correctly identified as positive and a positive identification was missed in 20 of the infected birds then the test has a sensitivity of 80%. The specificity of a test is defined as the ability of the test to correctly identify those animals that do not have the disease (true negatives). If we test the flock and 25 of the non-infected birds are correctly identified as negative and a negative identification was missed in 25 of birds then the test has a specificity of 50%.

In many cases sensitivity and specificity will not be available for wildlife species and may need to be estimated based on the sensitivity and specificity of the test in domestic animals. Information about sensitivity and specificity may be available directly from the manufacturer or in the manufacturers information provided with the test, or in articles by independent researchers who have evaluated the efficacy of the test in different species.

Note: The following equation/example is the method I suggested a pharmaceutical company use for determining appropriate sample size (Noordhuizen, et al) based on the desired probability of detecting disease. The equation can also be rearranged to determine the maximum prevalence or number of positives (d) in a population given that all individuals (n) tested negative. Unfortunately in a captive or translocation situation the sample size (n) is equal to the population size (N) if you set n = N the equation doesn't work because you can get a probability of detecting disease which is >1.

Equation for determining the probability of detecting disease based on sample size and test quality.

The likelihood of detecting disease in a population if disease is present is influenced by a number of factors including the total population size, the sample size, the sensitivity and specificity of the diagnostic tests being used, the expected prevalence of the disease in the population, and the "desired probability" of detecting the disease. Choosing a desired probability 0.95 or 95% means that if the disease is present, there is a 95% probability of detecting it and a 5% chance that it will not be detected. The following equation is one method of determining appropriate sample size (Noordhuizen, et al).

$$n = [1- (1-P)^{1/d}] [N – ((d-1)/2)]$$

n= sample size

P= probability of detecting at least one case if the disease is present

d= number of detectable cases in the population. If the diagnostic test being used does not have 100% sensitivity then d is equal to the number of infected animals multiplied by the sensitivity of the test. This assumes that no false-positives are present or that they are ruled out by confirmatory tests

N= population size

For example, suppose that we want to detect whether or not a flock of 300 birds (N=300) is infected with a specific disease. If the disease is present, then we suspect that the prevalence of disease is about 5% (i.e. about 5% of the birds will be infected) and the diagnostic test being used has a sensitivity of 100% (d=15 birds). We want to be 95% certain of detecting the disease (P=.95) which means that there is a 5% chance that we will be in error and will not detect the disease even if the disease is present. Based on the above calculation the required minimum sample size is 53 birds or 18% of the population.

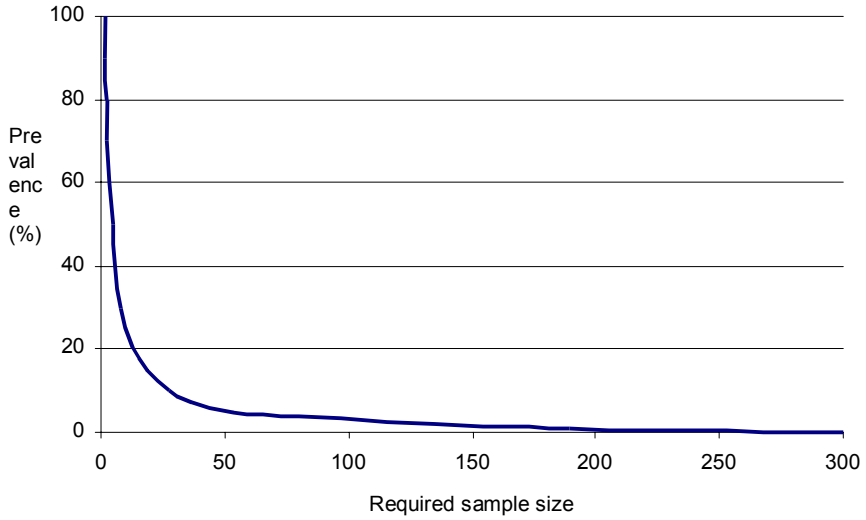
$$n = (1-(1-.95)^{1/15}) \times (300– (15-1)/2) = 53$$

If we assume that the test has a sensitivity of only 70% then it will detect 70% of the positive birds (d=15 x .7= 11 birds). Based on the above calculation the required sample size increases to 70 birds or 23% of the flock.

$$n = (1-(1-.95)^{1/11}) \times (300– (11-1)/2) = 70$$

The following table based on a flock size of 300 birds demonstrates how the required sample size changes as the prevalence of disease (Column A) changes. Columns C, D, and E give the minimum required sample sizes at different probabilities of detection.

Total number of birds in the flock	**Column A** Prevalence of disease in the population	**Column B** Number of diseased animals in the population	**Column C** Sample size required to have a 90% probability of detecting disease	**Column D** Sample size required to have a 95% probability of detecting disease	**Column E** Sample size - required to have a 99% probability of detecting disease
300	0	0	300	300	300
300	0.001	0.3	300	300	300
300	0.01	3	160	189	235
300	0.05	15	42	53	77
300	0.1	30	21	27	41
300	0.2	60	10	13	20
300	0.3	90	6	8	13
300	0.4	120	5	6	9
300	0.5	150	3	4	7
300	0.6	180	3	3	5
300	0.7	210	2	3	4
300	0.8	240	2	2	3
300	0.9	270	1	2	3
300	1	300	1	1	2



Ref: Kahn, H.A. and Sempos, C.T. 1989. Statistical Methods in Epidemiology, Oxford University Press, pp 12-42 and 230-244.

Noordhuizen, J.P.T.M., Frankena, K., van der Hoofd, C.M., and Gratt, E.A.M., 19 Application of Quantitative Methods in Veterinary Epidemiology pp 31-69.

ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

8. HEALTH ASSESSMENT WORKSHEET MANUAL

Sample Collection Appendix

THE COLLECTION,

STORAGE AND TRANSPORT OF

DIAGNOSTIC SAMPLES

FROM

BIRDS AND REPTILES

Compiled by:
Richard Jakob-Hoff, B.V.M.S. Veterinarian,
Auckland Zoo Wildlife Health and Research Centre,
Private Bag, Grey Lynn, Auckland

For : New Zealand Department of Conservation

July, 1999

CONTENTS

INTRODUCTION

These notes are intended to provide guidance to DOC staff faced with the need to collect diagnostic samples from native birds and reptiles in the field. This will usually be associated with a health screening or wildlife health surveillance project.

The role of the veterinarian

It is essential that a veterinarian with some background with bird or reptile medicine is consulted during the planning phase of your health screening or surveillance project. He/she will advise you on which diagnostic samples will be most valuable for your purposes and will interpret the laboratory results. With some prior notification many vets will also be happy to show you the correct technique for collecting samples.

To get the maximum value from your sample it must be collected, stored and transported in the right way and within the specified time frame. ***You should not go into the field collecting samples before you are trained to do so and have had the opportunity to practice***. This is particularly the case with blood and post mortem sample collection.

How the information has been organised

For easy reference a summary of the main points you need to remember is provided overleaf.

This is followed by a more detailed explanation of the methods of sample collection, storage and transport.

Additional information - including a glossary of terms - is provided in a series of appendices at the end of the booklet.

I. BLOOD - SUMMARY

Table 1: How much blood to collect and where from

	BIRDS	REPTILES
Max. Volume to Collect:	1% body weight (g) (1g = 1ml)	0.5 - 0.8% body weight (g) (1g = 1ml)
Collection Sites:	• Right neck vein (jugular) • Inside leg vein (metatarsal) • Wing vein (brachial)	• Tail veins

Table 2: Blood containers and their storage and transport

Sample type	Purpose	Container type	Storage	Transport
1. Blood smear	To assess general health including presence of anaemia, inflammation, infection, blood parasites etc.	Glass microscope slides with frosted ends	Air dried. Once air dried can be fixed with absolute methanol for 5 seconds, rinsed with water and air dried again - only necessary if > 4 days delay to lab. **DO NOT REFRIGERATE**	Plastic slide transport containers. These keep the slides dust-free, dry and prevent one slide touching another.
2. Very small blood volume (a few drops)	To measure packed cell volume (PCV) and total protein. Used to monitor general health, anaemia and dehydration	Glass capillary tube - range in volume from 9 - 60µl	Non-storable - must be centrifuged and processed in the field. Requires microcentrifuge and refractometer.	Not applicable
3. Serum or Plasma	For biochemistry tests to check health of internal organs and fluids. Also for measurement of antibodies against specific disease organisms.	Red-top (plain) microtainer (volume ≤ 0.5ml)	Clot at room temp. for 20 minutes then refrigerate. **CAN BE FROZEN** if centrifuged and separated from cells first.	In zip-lock bag within a box. Use chilly bin with freezer pack or ice blocks if delay to lab > 24 hours. Get to lab within 48hrs max.
4. Whole Blood	More accurate and comprehensive assessment of items listed under 1 and 2 above.	Purple top (EDTA) (preferable) or green top (heparin) microtainer. (volume 0.2 - 0.5ml)	Refrigerate/keep cool immediately. **DO NOT FREEZE**.	As for serum or plasma.

II. FAECES - SUMMARY

Table 3: Faecal collection, storage and transport

Sample type	Purpose	Container type	Storage	Transport
Faeces	Examination for parasitic worms (nematodes, tapeworms, flukes, coccidia etc.)	Clean plastic pottle. Can add equal volume of 5% formalin mixed thoroughly if unable to keep cool or delays > 3 weeks likely.	Refrigerate until sent to lab.	Place labelled containter in zip-lock BioHazard bag. To lab within 24 hours is preferred but even 3-4 week old samples can yield some results if kept chilled.
Faeces (very fresh)	Examination for fragile parasitic protozoa (e.g. *Cryptosporidia*, *Trichomonas*, *Giardia*)	Clean plastic pottle.	Send to lab immediately	As above. To lab within 1 hour is essential for these organisms. Refrigerate if any delay.
Faecal smear	For visual examination of gut bacterial flora, protozoa and presence of abnormal micro-organisms (e.g. yeasts).	Glass microscope slide with frosted end.	DO NOT REFRIGERATE. Air dry and keep at room temp. Can be heat fixed for longer preservation by passing slowly over a butane flame 5 times	In plastic slide container as for blood smears.
Cloacal flush	Any of the above if fresh faeces not available	Sterile glass or plastic pottle or tube.	Refrigerate/keep cool until sent to lab.	As for faecal/cloacal swab. Some protozoa may not be detectable after 1 -24 hours.

III ECTOPARASITES - SUMMARY

Table 4: Collection, storage and transport of ectoparasites (ticks, lice etc.)

Sample type	Collection	Storage	Transport
Ticks	Carefully pull off host with fine tweezers making sure mouthparts detach as well. Leaving mouthparts in skin can cause localised infection. A dab of alcohol on the tick can make removal easier.	Place in glass screw-top container containing 70% alcohol and 5% glycerol. Clearly label with 1) host species, 2) geographic area collected from, 3) body site collected from (head, ears, etc.), 4) date of collection and 5) name of collector.	Place container in plastic zip-lock bag, then outer solid container with packing to prevent breakage. To lab at your convenience.
Lice, mites	If unable to collect individuals with tweezers can briefly place body (not head!) of animal in a plastic bag containing a cotton ball lightly soaked in chloroform. Parasites will fall off and can be collected from bag.	As above	As above

IV. BACTERIAL CULTURE SWABS - SUMMARY

Table 5: Collection, storage and transport of swabs for bacterial culture

Sample type	Purpose	Container type	Storage	Transport
Faeces **Cloacal flush** **Cloacal swab** **Choanal swab** **(see below for collection technique).**	To identify gut or respiratory tract bacteria by culture	Paediatric swab in Stewart's transport medium +/- charcoal (latter preferable if transport delays > 24 hrs likely) OR Sterile screw-cap pottle.	Refrigerate/keep cool until sent to lab.	Place labelled swab in zip-lock BioHazard bag and courier bag in an outer container. If delivery delays likely (e.g. weekend) keep refrigerated or add ice to transport container.

V. POST MORTEM TISSUES - SUMMARY

Table 6: Collection, storage and transport of tissues for laboratory examination.

Sample type	Purpose	Container type	Storage	Transport
Preserved body organs including muscle, skin, bone etc. Take a small sample of **all** tissues. Samples of $5mm^3$ should be taken. If there is an obvious abnormality include this with an adjacent area of normal looking tissue**.**	Microscopic exam for signs of disease (histology)	Plastic container with screw cap containing 10% buffered formalin. The volume of formalin must be at least 10 times the volume of tissues. Clearly label with 1) Species 2) ID of animal 3) geographic area in which corpse was found 4) the date and 5) your name.	Tissues in formalin will last indefinitely and do not need refrigeration. However, **formalin fumes are highly toxic** so use only in well ventilated areas, seal containers tightly and store securely away from food, drink and areas of human or animal occupancy.	Seal container in a zip-lock plastic BioHazard bag. Place Wildlife Submission Form and a copy of your completed post-Necropsy Form (see below) in side pocket. Place bag in sturdy container with packing and courier to lab at your convenience.
Unpreserved tissues. Collect only if carcase is fresh. Take selected organs, particularly, liver, lung, kidney and any organ that appears diseased. A fairly large sample is useful	To look for and identify disease-causing bacteria in the laboratory.	Sterile plastic bag or screw-cap pottle.	Keep cool and refrigerate as soon as possible.	As above but place in separate zip-lock bag to preserved tissues. Formalin fumes from other containers may affect fresh tissues. Courier in chilly bin with ice and aim to get to lab within 24 hours of collection.
Frozen tissues Collect as for unpreserved organs.	To examine for viruses or toxic substances	As above	Keep in freezer. **NOTE**: THESE ARE THE ONLY TISSUES THAT SHOULD BE FROZEN	As for unpreserved tissues.
Body Fluids (e.g. urine, excess fluid in body cavities, blood etc).	To analyse for changes which may help to identify the cause of death.	Suck into a syringe with a sterile needle	Place into sterile tube or keep in syringe with needle capped. Keep refrigerated.	As for unpreserved tissues.

I. BLOOD SAMPLES

When to Take Samples

Ensure you are able to get the samples to a laboratory within 12 - 24 hours of collection ie. AVOID COLLECTING BLOOD ON FRIDAYS OR SATURDAYS if at all possible.

How Much to Take

COLLECT THE SMALLEST SAMPLE NEEDED FOR YOUR PURPOSES. This will vary depending on the size of the animal and what it is you wish to look or test for - check with your lab for the minimum amount they need. From a healthy reptile it is safe to take from 0.5 - 0.8% of their body weight. Up to 1% of body weight can be taken from a healthy bird. This represents 10% of their total blood volume. For example a 10g striped skink has a total blood volume of approximately 0.6ml, therefore the maximum blood you can take is 0.06ml while, from a 120g New Zealand dotterel you can take up to 1.2ml. Even the small amount taken from the skink is plenty for your vet to get a surprising amount of useful haematology and biochemistry data for health evaluation.

Blood Collection Sites

1. Reptiles

The tail (caudal) veins are the preferred sites in all native reptiles. These can be approached from the side, with the animal held on a flat surface in a normal resting position, or from underneath with the animal held on its back or held vertically with the tail hanging down. In all cases the tail must be straight out behind the animal and not curved round to one side. If collecting from the underside the site is in the midline about 1/4 the tail length back from the vent. If collecting from the side the site is the same distance from the base of the tail and just below the vertebral processes, the position of which can be felt with the fingers.

2. Birds

There are three main blood collection sites in birds but not all are accessible in all species.

The right neck (jugular) vein

This is the largest vein near the surface of the body and is the most suitable for collecting large samples quickly. In most birds there is a featherless tract of skin overlying this vein. This can be exposed by parting the feathers and then slightly moistening the skin with alcohol. Kiwis, pigeons and some other birds do not have this featherless tract and this vein should not be used in these species.

The wing (brachial) vein

This vein lies on the inside of the wing near the elbow joint and runs along the inside of upper wing bone (humerus). It can be seen by parting the feathers in this area and wetting with a little alcohol. Although this vein is readily accessible in most species (NOT kiwis) it is very fragile and likely to form a blood blister (haematoma) under the skin when the needle is withdrawn. Using small gauge needles, good restraint and pressure for at least 2 minutes after blood collection can help reduce this risk.

<u>The leg (metatarsal) vein</u>

This vein runs on the inside of the lower (unfeathered) part of the leg and can be seen or felt by pressure on the leg at the "hock" (tibiotarso-metatarsal) joint. This is the only site suitable in kiwis but is also an excellent site for waterfowl, waders, parrots and many other species. Because it is supported by the surrounding tissues it is less likely to form a blood blister than the wing vein.

Physical restraint

1. Reptiles

Even if you are very experienced at blood collection you should not attempt to do this on your own. Reptile veins are very small and fragile and any movement during collection will cause unnecessary damage and may result in excess blood loss. Therefore WORK IN PAIRS with one person doing the restraint and the other concentrating on the blood collection.

Also, remember ALL NATIVE REPTILES CAN SHED THEIR TAILS. Therefore, as the blood collection sites are in the tail, handle very carefully and avoid putting any traction on the tail itself. Placing one hand or a soft cloth over the animal's eyes will help to calm it during the handling. Similarly, when held on their backs, a gentle stroking with one finger along the length of the belly has a relaxing effect. Keep the handling to a minimum to minimise stress and the possibility of overheating.

2. Birds

As with reptiles, always have an assistant hold the bird for you while you collect blood. Method of restraint will vary with the species of bird and the site of blood collection. However, to avoid unnecessary trauma to the bird and its veins, restraint should prevent any movement. It is useful to use a flat surface (box, table top, the ground) to steady the bird against in most instances. ***Remember not to put any pressure on the chest as the bird needs to freely move this to breath***. Blindfolding the bird with a cupped hand, light towel, your armpit or other means will help to calm it and minimise stress. Avoid removing any more feathers than necessary to expose the blood vessel.

Blood Collection technique

1. Reptiles

With the animal suitably restrained the venipuncture site is cleaned of any dirt and then swabbed well with alcohol. A 25 - 27 gauge needle on a 0.5ml or 1.0ml syringe is advanced at a 70 - 90^{o} angle between scales into the animal until you just hit a vertebra. Apply a small amount of traction on the plunger and, at the same time, pull the needle back a bit until you see blood entering the syringe. Slowly pull back on the plunger until you have the desired amount of blood. *Do not take longer then 15 seconds or the blood will clot*. As the needle is withdrawn the assistant places a fresh gauze swab over the site and maintains steady pressure on it for at least 2 minutes.

2. Birds

Right Neck vein: The handler holds the bird's body with wings closed and legs held against the tail. The person doing the bleeding holds the bird's head and neck and exposes the vein as described above. The skin is swabbed with alcohol and the needle, with bevel side up, is introduced into the vein in the direction of the tail. Once the needle is in the vein it can help to turn the bevel side down before withdrawing the blood. The assistant can also help by placing a finger on the vein at the base of the neck. When blood collection is complete this finger is moved to a point just above the puncture site

for at least two minutes. Check carefully that bleeding has stopped before releasing the bird's head.

Wing vein: The handler holds the bird on its side against a flat surface with the upper wing, legs and head restrained. The lower wing, with its inner surface facing upwards is pulled out and held firmly by the person collecting the blood. Ensure this wing is held very firmly to prevent twitching at the time the needle is introduced. Swab the area of the elbow to expose the vein and introduce the needle in the direction of the shoulder. Use only a small amount of suction on the plunger as it takes very little vacuum for this vein to collapse. When blood collection is complete the handler places pressure over the vein with a dry gauze swab for at least two minutes.

An alternative method for collecting small samples from very small birds (e.g. robins) is to just prick the blood vessel and allow the drop of blood to fill a small capillary tube. Once full the end of the capillary tube can be touched against a microscope slide and a smear made with a cover slip as described below.

Leg vein: The animal is restrained on its side with both wings against the body, the head and upper leg held by the handler. The person collecting the blood holds the lower leg with inner surface facing upwards. The skin is thoroughly cleaned and swabbed with alcohol. The needle is introduced into the vein in the direction of the body. Blood is collected directly into a syringe or allowed to drip into a microtainer tube. Again, as with the wing vein, an alternative in small birds is to prick the vein and allow the blood to be sucked up into a capillary tube. When blood collection is complete, apply pressure with a dry gauze swab for at least two minutes.

Handling the fresh blood sample

Blood Smears

As soon as the needle is withdrawn place one small spot of blood on each of three microscope slides, near the frosted end. Make a blood smear by placing a 22 x 22mm cover slip on the blood spot in such a way that two corners of the cover slip overlap the slide. Allow the blood to spread out and then, in a single smooth action, spread the blood by sliding the cover slip along the length of the slide with your thumb and middle finger. Repeat with the other two slides in quick succession. Allow to air dry completely. DO NOT REFRIGERATE.

Blood Tubes

While the slides are drying the needle is removed from the syringe and the remainder of the blood placed gently in the tubes as described under anticoagulants above. Bird and reptile blood cells are VERY DELICATE so handle with care and do not force through the needle. If the cells break this will adversely affect your results. Refrigerate or place the blood tubes in a chilly bin until the samples can be sent to the lab.

Always replace the cap on the needle and dispose of it and the syringe in appropriate containers.

Labelling

It is vital that all diagnostic samples are clearly labelled with:

- **Species name**
- **Individual animal's ID**
- **Date of collection.**

Use a sharp pencil to label the frosted end of slides and a fine-point indelible pen or sticky label for the tubes. Clearly specify to the lab which examinations you want done and to whom the results (and the bill!) should be posted. Provide a fax and telephone number through which you can be contacted. All laboratories have submission forms which will make this an easier task for you so get a pad of them before you start. The samples can all be placed in special "Biological Hazard" zip-lock bags which have a pocket for the folded submission form. These can also be obtained through your lab.

Stopping the bleeding

In most cases simple pressure over the puncture site on a vein will stop the bleeding. Keep the pressure on for at least 2 minutes before checking. If bleeding has not stopped and the animal is not showing signs of severe stress (constant struggling, over-heating, vocalising, rapid respiration) apply pressure for a further minute. If this does not stop the bleeding or the bird is becoming severely stressed apply ferric subsulphate, silver nitrate or potassium permanganate (in that order of preference) to the site until bleeding stops. These chemicals will congeal the blood but also do some damage to the surrounding tissues so should not be used excessively.

Anticoagulants

These are chemicals that prevent blood from clotting. The two most common anticoagulants are heparin and EDTA. Both of them distort or damage blood cells to some extent. For this reason it is best to make blood smears without any anticoagulant as soon as the sample has been collected.

For the remainder of the sample remove the needle and gently empty it into one or more of the following Microtainer tubes:

Purple top: This contains potassium-EDTA and you will need between 0.2 - 0.5ml of blood to get the right anticoagulant to blood ratio. Run the blood down the side of the tube, snap on the top and gently roll it to mix the blood and anticoagulant. EDTA gives better preservation of the blood cells and allows more accurate blood counts than heparin. If it is necessary to make your own EDTA a 30% solution can be made by mixing 30g EDTA with 100ml sterile distilled water. A minute amount is needed to prevent clotting of up to 0.5ml blood. Draw up a small amount to coat the inside of the syringe and then expel all excess by repeatedly pumping the plunger in and out.

Green top: This contains heparin. This tends to interfere with staining of the blood cells but, in combination with fresh smears, will enable most haematological exams as well as several biochemical tests on the plasma. Blood should be gently placed into the container and mixed as with the purple top.
As with EDTA a small amount of sodium- or lithium-heparin can be used to coat the inside of a syringe. 25 units of heparin will prevent clotting in up to 1ml of blood. Remember to collect blood smears without anticoagulant for best results.

Red top: This has no anticoagulant in it. It is used when your objective is to get serum biochemistry or serology (antibody) tests. Even a very small amount of blood, when allowed to clot in these tubes, will yield enough serum for several biochemistry tests. Blood is gently placed into them but not agitated. Leave to clot at room temperature for 20 minutes and then refrigerate.

Storage of blood samples

As mentioned, **red top tubes** should be allowed to clot for about 20 minutes at room temperature and then refrigerated. **Purple top and green top tubes** should be refrigerated immediately. If you are not near a fridge place the samples in a chilly bin with ice or freezer blocks. Place newspaper or bubble wrap between the sample and the ice to prevent freezing. With whole blood in tubes that can be kept cool in a fridge or chilly bin you should aim to get them to the lab within 24 hours (e.g. avoid collecting samples on a Friday when their processing is likely to be delayed by the weekend). However, where delays can't be avoided, a 48 hour interval between collection and arrival at the laboratory is acceptable provided the samples are kept continuously cool in the interim.

If you are able to separate the **plasma or serum** from the RBCs by sedimentation or centrifugation then this can be safely frozen and kept for weeks or months. Plasma or serum can be used for biochemistry and serology but not haematology or DNA tests.

Blood smears should not be refrigerated at any time as they must be kept dry. Air dried blood smears, held in dust-proof plastic slide boxes at ambient temperature will remain viable up to 4 days. If you are unable to get them to a lab in that time frame, the smears can be preserved ('fixed') by immersing them in absolute methanol for 5 seconds, rinsing with water and letting them air dry again. You should let the lab know you have done this when you send them. Ensure slides are kept separate from each other, i.e. do not stack one directly on top of another.

Transport of blood samples

Place all samples in BioHazard zip-lock bags available through your lab. The completed laboratory submission form should be placed in the pocket of this bag. As long as all samples are clearly labelled several can be placed in the same bag.

Slides containing blood smears should be placed in plastic slide containers which can hold from 1 to 5 slides, each separated from the other. If you are going to collect samples in the field make sure you take several of these containers. Make sure the slide is fully air-dried before placing in the container.

Whole blood in tubes can be placed with the slide container in the zip-lock bag and the whole bag placed in a cardboard or plastic box if it is going to get to the lab within 4 hours. If a delivery delay of more than 4 hours is likely send the bag with some paper or bubble wrap packing in a chilly bin containing ice or freezer blocks.

Ensure your container has the laboratory street address and telephone number clearly marked on the outside as well as your contact details. It is a good practice before you collect your samples to let the lab know, particularly if there are to be a large number or you are requesting an out-of-the-ordinary test. They may give you special instructions on how they would like the sample collected, stored and sent.

II. FAECAL SAMPLES

Selecting the sample

Birds and reptiles evacuate all their wastes together. Each dropping is made up of three components:

1. Faeces - the solid, dark part
2. Urates - the solid or semi-solid white part
3. Urine - the clear liquid part (usually not visible under field conditions).

When collecting a faecal sample try to avoid also taking the urates or any substrate (not always easy or possible!).

If collecting from a group of animals, collect several samples that you feel are representative of the group into the same container.

Old faeces (≤ 1 week old), providing they haven't completely dried out, can still contain recognisable stages of some parasites e.g. nematodes, tapeworms, flukes and coccidia. These stages are adapted for survival in the environment for lengthy periods.

Fresh faeces (≤ 1 hour old) are needed to identify the more fragile parasites, particularly the mobile protozoa such as *Giardia*, *Trichomonas* and *Cryptosporidia*.
Swabs of fresh faeces are also necessary for bacterial culture.

A sample of **1 - 5g** is adequate for all laboratory faecal examinations. Use a clean spatula to place the sample into a clean, plastic container. If the sample is for bacterial culture it must be placed in a sterile pottle.

SAFETY NOTE: All birds and reptiles can harbour *Salmonella* and other disease organisms which could make you sick. Therefore always handle faecal and other samples hygienically **and wash hands thoroughly with undiluted hibiclens[*] (or similar antiseptic) after handling animals or their wastes**.

Special collection techniques

Faecal smears and bacterial culture swabs:

Faecal smears can be a very valuable health indicator and can also uncover the presence of abnormal organisms such as yeasts (e.g. *Candida*). To make a smear lightly dip a sterile swab into the top of a fresh faecal sample. Gently smear this along the centre of a glass microscope slide and allow to air dry. Place the swab into the swab container. Label the swab and slide.

Cloacal flush

A cloacal flush can be performed on both birds and reptiles if you are unable to obtain a faecal sample. The procedure can be done under manual restraint and is the same in both groups of animals. An appropriately sized smooth, plastic catheter is lubricated (e.g. with KY jelly) and attached to a syringe containing luke-warm 0.9% sterile saline (the volume of fluid should be no more than 1% of the animal's body weight). The catheter is gently passed into the cloaca being careful not to force it. The saline is then pushed into the cloaca and sucked out again. Repeat this 3 - 4 times and then remove the catheter. The fluid is placed into a sterile container, labelled and forwarded to the laboratory.

Faecal sample storage

Whole faeces and cloacal washes

Keep your sample in a closed container in the fridge or in a chilly bin containing ice. DO NOT FREEZE. As mentioned some parasites (e.g. Giardia) will not be detectable if there is a delay of > 1 hour in getting it to the laboratory.

Faecal preservation

[*] Hibiclens Skin Cleanser antiseptic (= 4% chlorhexidine gluconate), ICI pharmaceuticals.

Keep cool in fridge if possible. If there are likely to be delays of more than 3 weeks in getting faeces to a laboratory you can add 5% formalin to the faeces to preserve most parasites (check with the lab first). Add a volume of formalin equal to the volume of faeces and mix thoroughly with a clean instrument. If you are looking for the more fragile organisms you should consult your lab for advice on handling the faecal specimens you collect.

Faecal smears

Faecal smears should be allowed to completely dry at room temperature before being placed into a slide container for transport. If there is likely to be a delay of >24 hours in getting the slide to the lab you can "heat fix" the bacteria by slowly passing the slide through a butane flame 5 times. Advise the lab that you have done this. DO NOT REFRIGERATE.

Faecal sample transport.

Place faecal pottle and slide container in a zip-lock BioHazard bag and pack this into an outer package. Use a chilly bin with ice if the sample is for culture or identification of protozoa and delays of >1 hour are likely.

III. ECTOPARASITE SAMPLES

Some ectoparasites, such as mites are very small and difficult to see unless they are moving under a bright light. Also many mites are active only at night (e.g. red mites) when they come and suck blood from their hosts. They spend the day time hiding in cracks and crevices in the environment where the animal lives. Therefore, if you suspect mites are causing a problem but can't see them on the animal, check the environment where the animal spends time at night (e.g. roosting sites). Some dust samples from crevices in this area placed in 70% alcohol with 5% glycerol may uncover the culprits. (If you do find mites, be wary about jumping to conclusions - most mites live in soil, are completely harmless and may not be the cause of your problem!).

It is useful to identify the species of ectoparasites because this yields information on their life cycles which can be used by your vet to recommend the most effective treatment and control measures.

Ectoparasite collection techniques

Ticks

Using fine-tipped tweezers with a curved end grasp the tick under the head to extract its mouth parts. Failure to remove the mouthparts can cause irritation and localised infections. If the animal is severely covered in ticks you may need to remove them over

2 -3 days to prevent excessive trauma. Applying a dab of alcohol to the tick relaxes it so that it can be removed more easily.

Lice and Mites

Some lice and mites can be collected with tweezers or by touching them with a water-moistened wooden applicator stick (avoid using cotton buds as the parasites get tangled up in the cotton strands). If this is unsuccessful the animal's body (but not head!!) can be placed in a plastic bag containing a cotton ball or gauze swab lightly soaked in chloroform. DO THIS IN A WELL VENTILATED SPACE. The animal is restrained for 1-2 minutes and the bag held closed around the neck to avoid inhalation of the chloroform fumes. Remove the animal and dispose of the chloroform swab. The parasites will have fallen into the bag and can be placed directly into your storage container.

Ectoparasite storage

The parasites are placed into a solution of 70% alcohol and 5% glycerol in a screw-capped container which is labelled as follows:

1. Species name of host animal
2. ID of individual animal
3. Geographic site of animal collection
4. Body part from which parasites collected (e.g. external ear canals)
5. Date of collection
6. Your name and contact number

No refrigeration is required.

Ectoparasite transport.

Place the container in a zip-lock bag then outer, solid container with packing, if necessary to prevent breakage in transit. Can be forwarded to lab. at your convenience.

IV. BACTERIAL CULTURE SWABS

Bacterial cultures are used to identify disease-causing bacteria. Once cultured they can also be tested against various antibiotics to see which are the most effective. This is valuable information used to guide treatment where necessary.

Collection techniques for bacterial cultures

Faeces

This was described under faecal sample collection techniques

Cloacal flush

Also described under faecal sample collection technique. However, remember that, for bacterial culture the sample must be collected with a sterile catheter and sterile saline and placed in a sterile container.

Cloacal swab

The area around the vent is lightly cleaned with alcohol. A paediatric swab is moistened by dipping it into its transport medium and then inserted gently into the cloaca. **Do not force or you will inflict damage**. Lightly rotate and then withdraw and place into the transport medium.

Choanal swab

The choanal slit is an opening in the roof of the mouth which connects directly with the nasal cavity. This is a good site from which to culture bacteria if a disease of the upper respiratory tract is suspected. In some birds (e.g. pigeons) the bill is easily held open with fingers while with others (e.g. parrots) and most reptiles an instrument is needed to do this. This could be a pair of tweezers, forceps or sturdy bent (but blunt ended) wire. Be careful not too damage the mouth or bill or to use something that the animal could bite and swallow or injure itself.

Once the mouth is open direct the tip of the swab into the choanal slit towards the nostrils and swab from front to the back. Slightly rotate the swab as you do your sweep. Place in the transport medium and label.

Storage of culture swabs

Bacterial culture swabs are sold in sterile packets that include a sleeve containing Stewart's transport medium into which the used swab is placed. This medium prevents the swab from drying out in transit and provides an environment suitable for most types of bacteria. Some can be purchased which include charcoal in the medium. This absorbs bacterial toxins and is particularly useful if there are likely to be delays in transporting the swab to the laboratory.

Transport of culture swabs

The swab in its transport medium should be placed in a zip-lock BioHazard bag and refrigerated or kept cool until it can be sent to the lab. Ideally it should reach the lab within 24 hours. If this is not possible (e.g. due to weekend) keep refrigerated until the first available mailing day.

V. POST MORTEM (NECROPSY) SAMPLE COLLECTION

If it is possible to send a dead animal directly to a lab or veterinarian for post mortem this will get you the best results. If there are likely to be delays in shipment and the animal is small, open the body cavity and place the entire animal into 10% formalin to prevent further decomposition. Ask the lab to do a post-mortem on this preserved specimen. If this is not possible it is better to have a go yourself rather than leave the carcase to rot. The following outlines a procedure for performing a post mortem on a bird or reptile. It is essential to be systematic and follow the same procedure each time to avoid missing things.

Before You Start

1. Make sure you have the equipment listed in Appendix II and a clean, dry, solid surface to work on. The equipment must be clean and cutting instruments sharp.

2. Ensure your personal safety from contagious diseases (e.g. psittacosis, Salmonella) - wear rubber gloves, a face mask and overalls or other garment to protect your clothes from contamination. Work in a well ventilated area with good lighting.

3. Fill out the baseline information in a Necropsy Form . This should include:

    - Specific details relating to the animal:
      species, individual ID (e.g. band or microchip number), age (even "juvenile" or "mature adult" is better than nothing), sex, location where animal was found dead, the date the animal was found.

    - Case history: a summary of any information you have relevant to this animal and its circumstances of death.

    - Your details: Name, organisation and contact details

    - The date of the post mortem.

    - Note all your findings as you proceed.

External Examination

Record the body weight and standard measurements (e.g. snout to vent, bill length etc).

Before cutting into the animal carefully check the entire carcase for body condition and state of decomposition. Check for any abnormalities such as missing scales or feathers, wounds, swellings etc.

For birds, once you have checked the plumage, it is best to remove all the feathers taking care not to tear the skin. This has two benefits:
1) it prevents messy feathers getting in the way when you cut into the bird and

2) you will get a much better appreciation of the condition of the skin as a whole and the presence of any bruises or small wounds.

Once the feathers are removed briefly dip the carcase in water or under a running tap to damp down any remaining down feathers.

Internal examination

Lay the animal on its back on your dissecting board and cut the skin from the vent to the jaw. In birds take care not to cut into the crop which lies very close to the skin surface on the right side of the neck. From this midline incision cut across to each limb and then dissect off the skin to the sides of the body.

Carefully remove a flap including the abdominal muscles, ribs and and keel (sternum) to expose the internal organs. Do this slowly and carefully taking note of any excess blood or other fluids which may escape from the body cavities. In birds also carefully check the air-sacs which will be ruptured as you remove this flap - they should be thin and transparent.

Examining the organs
Before you remove any organs check that they are in their normal positions and note any abnormalities. It takes some time and practice to get to know the normal appearance of organs in the different species.

Remove and examine the organs in the following order:

- Heart
- Liver
- Thymus (present just above the heart)
- Thyroid glands (at the base of the trachea)
- Lungs and trachea
- Pancreas (usually visible within the first loop of intestines before you remove the gut)
- Gastrointestinal tract from the mouth to the vent
- Spleen (usually close to the stomach, small, spherical and red)
- The reproductive tract including ovaries or testes.
- The adrenal glands (which lie close to the gonads and kidneys)
- The kidneys (lie in a depression in the pelvis or within the pelvic canal)
- The eyes
- The brain
- The muscles and skeleton including joints, claws, beak etc

Once you have removed all the organs examine each one for changes in:

- **Size**
- **Shape**
- **Colour**
- **Consistency**

and make appropriate notes on your Necropsy Form. If you have the opportunity to weigh any of the organs this is also useful information. Cut into each organ and check for any of the above changes. Also note any contents in the stomach and intestines and the presence of parasites.

Taking samples for laboratory examination.
As you examine each organ place a small sample (about 5mm square) in 10% formalin. Where you suspect gout (chalky white deposits around joints or within the body cavity or kidneys) place the affected tissues into 70% alcohol which, unlike formalin, doesn't dissolve the uric acid crystals.

WHERE YOU SEE ABNORMALITIES ALWAYS TAKE A SAMPLE!

If you suspect toxicity or viral disease or you want tissues for DNA work it is worth keeping some samples frozen. Place parasites in separate, labelled small containers in alcohol or 5% formalin. Remember to write down all the samples you have collected on your Necropsy Form and label all containers clearly.

Samples for bacterial culture need to be from fresh, unfixed tissues placed in a sterile container. Provided you haven't contaminated them with your instruments, you can also take swabs for culture from fluids, abscesses etc. or you can collect some of the fluids or pus into a sterile syringe and needle and send this, with the needle capped, to the lab for culture.

Storage of Necropsy Samples
Fixed samples should be stored at room temperature in tightly sealed screw-cap containers.

Fresh, unfixed samples, for bacterial culture (including swabs, fluids, pus, tissues etc) should be stored in similar sterile containers and kept in a refrigerator or chilly bin with ice.

Fresh, unfixed samples for viral culture or toxicology should be placed in a similar sterile container and kept frozen.

Transport of Necropsy Samples.
Ensure all sample containers are well sealed and place them into zip-lock biohazard bags. These can then be placed in a plastic or cardboard sealed container with appropriate packing to prevent movement during transit.

Fresh or frozen samples should be placed in a chilly bin with ice or freezer packs.

Aim to get the samples to the laboratory within 24 hours of collection.

APPENDIX I

GLOSSARY OF TERMS

While the use of technical jargon has been minimised it is not possible to describe diagnostic tests without the use of some of the more commonly used terms. The following definitions have been used in this booklet.

Biochemistry	The scientific study and evaluation of blood chemicals
Erythrocytes	An alternative name for red blood cells (RBCs)
Haemaglobin (Hb)	The red pigment used in red blood cells to transport oxygen
Haematocrit (Hct)	An alternative name for packed cell volume (PCV)
Haematology	The scientific study and evaluation of blood cells
Leucocytes	An alternative name for white blood cells (WBCs)
Microbiology	Examination of diagnostic samples for bacteria
PCV	Packed cell volume, a measure of the relative proportion of cells vs fluid in blood; used as a measure of anaemia and hydration status
Plasma	The fluid component of blood before the cells have clotted.
RBC	Red blood cells containing the pigment haemaglobin (Hb)
Refractometer	An instrument used to measure serum protein concentration
Serology	The measurement of antibody levels in serum
Serum	The fluid component of blood after

	the cells have clotted.
Thrombocytes	Small blood cells involved in blood clotting (called platelets in mammals)
WBC	White blood cells, a key component of the immune system
Whole blood	Blood containing an anticoagulant before the plasma has been separated from the cells

APPENDIX II

EQUIPMENT FOR DIAGNOSTIC SAMPLE COLLECTION

1. General

• Gauze swabs • Sharp pencil • Indelible fine-point black pen • Adhesive labels • Pre-labelled sample transport containers • Packing tape to seal transport container. • Courier labels • Tissue paper or bubble wrap for packing samples • Plastic slide transport containers	• Rubber gloves • Hibiclens antiseptic hand wash • Plastic zip-lock "BioHazard" bags • Plastic rubbish bag or box for used needles etc. • Pad of laboratory submission forms • Ice blocks or freezer packs as needed • Chilly bin • Bag, towel or soft cloth to blindfold animal during restraint as appropriate • Scales to obtain body weights of animals

In addition to the above the following specific equipment is useful:

2. Blood

• 0.5 or 1.0ml syringes • 25, 26 or 27G hypodermic needles • Alcohol swabs • Absolute methanol for fixing blood smears (if delay > 4 days in getting to lab) • Silver nitrate sticks to stop bleeding if necessary	• 10 µl x 32mm capillary tubes • Microhaematocrit centrifuge • Refractometer • Microscope slides with frosted ends • 22 x 22mm cover slips • Microtainer blood tubes (purple, green and red-top)

3. Faeces

• Clean screw-cap plastic pottles • Sterile plastic or glass pottles • 5% formalin • Butane lighter • Tongue depressors or similar spatulas	• Sterile smooth, round-ended plastic catheters • KY jelly • 0.9% sterile saline • Microscope slides with frosted ends.

4. Ectoparasites

• Fine tweezers with curved tip • 70% alcohol/5% glycerin solution • Screw cap pottles • Wooden applicator sticks	• Chloroform • Cotton balls • Plastic bags • Bright torch

5. Bacterial Culture Swabs

• Paediatric culture swabs in Stewart's transport medium with charcoal	• Sterile screw-cap pottles.

6. Post-Mortems

• Sharp, clean instruments (No.3 scalpel handle, No. 15 scalpel blades, small and large scissors, toothed and plain forceps) • 10% buffered formalin • Plastic screw-cap pottles and/or sealable plastic buckets • Sterile plastic bags and/or screw cap pottles	• Overalls or similar protective clothing • Face masks • Sterile 1ml and 3ml syringes • Sterile 23 and 25 gauge needles • Necropsy Forms • Wildlife Submission Forms

APPENDIX III

THE VALUE OF BLOOD AS A DIAGNOSTIC TOOL

Introduction

Because it circulates throughout the whole body and is readily accessible blood is one of the most useful tools for health evaluation. Veterinary diagnosis is based on detective work - piecing together a number of clues in order to see the "big picture" of what is really going on. It is very rare for any one diagnostic test to give you an answer, more often its a case of looking at a variety of clues, weighing up the relative significance of each and then coming to your diagnosis. For this reason you will need the assistance of a veterinarian to help you select and interpret the specific blood tests appropriate to your situation. However, the following is included to give you a basic understanding of blood and how it can be used as a diagnostic tool.

Composition of blood

Blood is composed of **cells** suspended in a fluid called **plasma** which constantly circulates throughout the body. The cells are either red blood cells (RBCs or erythrocytes), white blood cells (WBCs or leucocytes) or thrombocytes.

Red blood cells

There is only one type of RBC and its main function is the transport of oxygen to the tissues via an iron-containing protein called haemoglobin (**Hb**). The proportion of RBCs as a percent of total blood volume is called the Packed Cell Volume or **PCV** (also sometimes referred to as the haematocrit or Hct). In birds this generally varies from 35 - 55% while in reptiles the range is generally 20 - 35%. Because of the vital role of RBCs the measurement of the PCV and Hb concentration are very valuable indicators of health. Different disease processes also effect the size, shape and internal structure of RBCs all of which can be assessed by an experienced eye from a stained blood smear. RBCs can be invaded by some blood parasites (e.g. *Plasmodium*, the cause of avian malaria) and these can be identified with special stains.

White blood cells

WBCs are part of the immune system and are therefore involved in body defences.

The following types of white blood cells are found in birds and reptiles:

Heterophil	This is usually the most numerous WBC. It has a role in inflammation and the first line of defence in acute bacterial infections.
Lymphocyte	This cell is also involved in inflammation, but usually of a more long-term nature. Some types produce antibodies, others invade the sites of infection in the tissues, often with monocytes.
Monocyte	These are the largest WBCs and are involved in combating chronic

	infections by cell-invading organisms such as viruses, protozoa, chlamydia and TB bacteria.
Eosinophil	These are usually present in low numbers except when an animal is suffering from parasitism or an allergy.
Basophil	These are usually the rarest WBC and also have a role in combating allergens. When they leave the circulation and enter tissues they convert to mast cells which produce histamines.

A total WBC count is made from a blood sample containing an anticoagulant (see below) and is a valuable but non-specific indicator of the presence of inflammation, infection, stress and, in some cases, cancer (e.g. leukaemia). A differential WBC count is made from a blood smear and assesses the total number and proportion of each type of WBC. This allows the veterinarian to narrow down the possible types of disease processes going on. Some blood parasites (e.g. leucocytozoon) can also invade WBCs and can be identified from a specially stained blood smear.

Thrombocytes

Thrombocytes are the equivalent of the mammalian platelet. They are involved in blood clotting and are consumed when there is active inflammation. Their numbers are assessed in the blood smear.

Plasma

As mentioned plasma is the non-cellular part of blood. It is a very complex fluid that contains a wide range of proteins which are involved in body defence (eg antibodies), control (e.g. enzymes, blood clotting factors and hormones) and transport of chemicals around the body (e.g. albumin). **Albumin** is the most important blood protein for regulating the animal's fluid balance. **Globulins** are the other major protein and are made up of antibodies. A rise in globulins indicates an active infection. The measurement of both is called the Total Protein (**TP**) and is a useful indicator of hydration status and the presence of disease.

Enzymes are essential catalysts of chemical reactions that occur in the body. Different enzymes are localised within different cells in the body. When these cells are damaged the enzymes leak out into the blood where their concentration can be measured in the plasma. A rise in enzymes can help localise the site of disease. Common enzymes used to assess damage in different organs are:

Muscle and heart:	Aspartate aminotransferase (**AST**) and Creatine Kinase (**CK**)
Liver:	Lactate dehydrogenase (**LD** or **LDH**) (also elevated by RBC breakdown (haemolysis).
Pancreas:	**Amylase**, **lipase**

The plasma also contains nutrients (e.g. glucose) minerals (e.g. calcium), electrolytes (e.g. sodium, potassium) and the end-products of the body's metabolism. These may also be raised or lowered in the presence of disease. Those of diagnostic value in birds and reptiles include:

Kidney:	**Uric Acid**, the equivalent to mammalian urea which is a waste product from protein metabolism. It can cause serious damage (gout) when elevated in the presence of kidney disease or dehydration.
Muscle, heart	**Potassium** (also elevated by haemolysis)
Liver:	**Bile acids**
Bone:	**Calcium**
Pancreas, liver	**Glucose**

Serum

Serum is the non-cellular part of blood after it has clotted (i.e. when no anticoagulant is used). Because it is devoid of clotting factors Total Serum Protein (**TSP**) will be slightly lower than the total protein (**TP**) measured from plasma in the same animal. However, serum can be used to evaluate all the factors listed above.

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ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

9. MODELING OF POPULATION AND DISEASE DYNAMICS: *OUTBREAK* and *VORTEX*

OUTBREAK
A Model of Wildlife Disease Epidemiology and its Impacts on Population Viability

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Project Summary

Population viability analysis (PVA) has become a valuable tool in the arsenal of conservation biologists as they seek to develop more effective ways to manage and conserve endangered and threatened wildlife species. Presently, there are several good simulation packages of wildlife population dynamics that incorporate detailed models of the diverse deterministic and stochastic (random) forces affecting the growth or decline of populations that are adversely impacted by human activity. In addition, there is a group of several informative epidemiological models that biologists employ to simulate disease spread through wildlife populations. However, the available population viability analysis models use, at best, only simplistic representations of the role of disease in wildlife population dynamics, while the epidemiological models assume static population size or use only very simple models of population change. Yet the dynamics of both population change and of diseases may be found to be very different if we were to assess simultaneously a more inclusive range of stochastic processes that affect each, perhaps in complex and interacting ways. In response to this need to develop a more comprehensive tool for PVA, we propose to construct a detailed integrated simulation package that will allow modeling of intertwined wildlife population dynamics and wildlife disease. This simulation modeling environment will no doubt improve our understanding of both systems and, therefore, will promote a closer collaboration between wildlife population biologists and veterinarians as they work to conserve our planet's biological diversity.

Statement of Research Problem

Conservation biologists use a tool known as population viability analysis (PVA) to evaluate the risk of wildlife population extinction resulting from human impacts on the environment. While disease can be a major factor influencing the survival of threatened populations, traditional PVA methods treat the complex nature of disease in very simple terms. Existing models that simulate the nature of infectious disease have not been adequately and systematically linked with PVA applications in wildlife conservation.

Specific Hypotheses and Objectives

Hypotheses:

1. Integrating quantitative simulation models of infectious disease epidemiology and wildlife population viability will lead to a greater understanding of the dynamics of disease and its role in impacting population growth.
2. Greater understanding of the interaction between wildlife disease epidemiology and population growth dynamics will lead to more informed and effective conservation of threatened and endangered biological diversity.

Objectives:

1. To develop a detailed, individual-based model of disease epidemiology for use by the global wildlife disease ecology and veterinary community.
2. To develop a detailed integrated simulation package that will allow individual-based modeling of intertwined wildlife population dynamics and disease epidemiology.
3. To develop a tool that can be used for evaluating disease risks associated with wildlife conservation strategies and for comparing the costs and benefits of disease prevention and control programs in wildlife populations.

Justification and Significance

While substantial effort is being directed toward constructing demographic models of wildlife population viability with greater realism and mathematical sophistication (e.g., Sjögren-Gulve and Ebenhard 2000; Reed et al. 2002; Beissinger and McCullough 2002), there is considerably less attention directed at the larger ecological factors that influence population persistence. One such factor is infectious disease and its transmission dynamics among co-existing human and animal populations. PVA models do not adequately reflect the demographic effect of disease on a population, which can vary considerably depending upon the structure of the host population, the characteristics of the infectious agent, and environmental variables such as habitat condition and availability.

Interestingly, the great majority of epidemiological models of infectious disease focus primarily on the disease status of the individuals in the population (e.g., susceptible, infected, recovered) and assume a static population size or use only very simple models of population change. As a result, these models produce estimates of morbidity and mortality without considering the important effects of random demographic, environmental, and genetic factors. In other words, they lack the core components that make effective PVA models inherently useful: an explicit treatment of the intrinsic and extrinsic stochastic forces that put small populations of wildlife at risk of extinction. By developing a detailed, individual-based simulation modeling package of the epidemiology of wildlife disease, and by studying the impacts of disease on population viability through its linkage with an existing PVA model, we will be able to greatly enhance our understanding of 1) the process of disease transmission in small wildlife populations subject to unpredictable demography (i.e., birth and death rates), and 2) the interactions that occur among demographic factors, environmental variables, disease pathogens, and host genetics to impact endangered population persistence. This integrated product will be an extremely valuable tool for evaluating disease risk in wildlife conservation strategies and will provide an outstanding opportunity for productive collaboration between the wildlife ecology and veterinary communities. Moreover, the package will serve as a unique teaching vehicle for students in these fields of study. Through this process, our effort will also promote a more intimate integration of scientific disciplines – an endeavor that has

recently been argued as vital to the success of biodiversity conservation and its inherent complexity (Redman 1999; Nyhus et al. 2002; Lacy and Miller 2002).

Literature Review

An Introduction to Population Viability Analysis

Under almost any set of circumstances, wildlife populations will fluctuate in size over time. These fluctuations result from random (stochastic) variation acting on a set of processes – most notably birth, offspring sex ratio, dispersal, and death – that, acting together, determine the dynamics of population change. Numbers of individuals comprising a given population are determined largely by specified rates of reproduction, survival, and dispersal in addition to the ecological limitations of habitat carrying capacity. Variation in these rates over time is influenced by processes both intrinsic (demographic stochasticity, genetic drift and/or inbreeding depression, or deviations in age or social structure) and extrinsic (environmental variation and catastrophic events) to the population (Shaffer 1981; Soulé 1987).

While random fluctuations in size are a normal part of wildlife population dynamics, reductions in mean population size brought about by human activities can result in a greatly increased risk of extinction through annual stochastic variation in demographic rates. As a population declines in size, these random forces can produce larger proportional changes in annual population size. The process can continue over time until a single major event or an unfortunate set of events occurring simultaneously can eliminate the population. This synergistic interaction between population size and stochastic extinction risk is summarized in the heuristic "extinction vortex" model introduced by Gilpin and Soulé (1986). The recognition that threatened populations could become extinct largely through bad luck – even in the presence of active management designed to increase population size over the long term – was a major advance in the emerging multi-disciplinary field of conservation biology.

Population viability analysis (PVA) soon emerged as a method for practical application of the extinction vortex concept by examining the threats to persistence of wildlife populations (Boyce 1992; Beissinger and Westphal 1998; Groom and Pascual 1998). Traditionally, PVA starts with a model of the forces that drive population change and then assesses population performance under a specified set of conditions. PVA can use empirical, analytical, or simulation methods, but most processes rely on simulation to assess the interacting affects of a large number of complex processes. Individual-based models are the most appropriate tool for investigating extinction dynamics in small populations, particularly when including inbreeding depression and other random genetic processes (Lacy 2000a). The primary use of PVA is to estimate the probability of extinction of a population, the mean time to extinction, or other measures of population performance such as growth rate, stability, or genetic diversity. A comparison of such measures of population viability for a set of different scenarios then allows comparison of which threats are most important. In addition, sensitivity analysis can be used to determine the primary demographic determinants of population growth (e.g., Wisdom and Mills 1997), and management alternatives can be compared to determine the most effective conservation strategies (e.g., Possingham 1995; Hamilton and Moller 1995; Herrero et al. 2000). In a recent comprehensive study of the predictive capability of PVA modeling packages, Brook et al. (2000) demonstrated that, when adequately parameterized with reliable field data on the species or population of concern, PVA methods can provide a reliable technique for demographic population projection. Studies such as this have bolstered the validity of PVA as a predictive tool; however, when population data are scarce or when models are not sufficiently detailed, quantitative predictions of the fate of endangered wildlife populations should be interpreted with caution. In these cases, comparative analyses such as those described above constitute the most appropriate use of PVA methodologies.

Disease Epidemiology Models and PVA

An extensive literature exists on the theory of infectious disease ecology in natural populations of wildlife (Grenfell and Dobson 1995) as well as humans (Anderson and May 1982, 1991). The seminal papers by Anderson (1982) and May (1986) laid the conceptual foundation for how we discuss infectious disease dynamics in natural populations. These early publications formed the basis for a more detailed treatment of both theoretical (e.g., Li et al. 1999) and applied (e.g., McCarty and Miller 1998) infectious disease dynamics. Most of these analyses include so-called "S-I-R" – type models of disease transmission dynamics that describe the relative proportion of a population comprising each disease state – susceptible (S), infectious (I), or recovered (R) – and the time-dependent probabilities of transition between states based on the specific characteristics of the infectious disease under study. Important characteristics include the disease prevalence, the contact rate between individuals in the population, the probability of transmission of the infectious agent given a contact, the latent period of infection, the disease-specific mortality rate, the rate of acquisition of resistance.

The agricultural community has repeatedly applied components of this theoretical background in quantitative risk assessments associated with, for example, the movement of animals and/or animal products (e.g., MacDiarmid 1993; Clement et al. 1995). Some of these analyses have demonstrated specific relevance to the field of biodiversity conservation *in situ* (e.g., Hess 1996), and the wildlife veterinary community is beginning to develop an increased awareness of the importance of quantitative risk assessment (e.g., Woodford 1993; Armstrong and Seal 2001). However, as pointed out nearly a decade ago by Lyles and Dobson (1993), the large majority of conservation biologists ignore wildlife population disease concerns outright or, at best, treat them in a very cursory fashion. Comparatively, consideration of disease in the captive propagation of wildlife species has received significantly more attention (e.g., Wolff and Seal 1993; Ballou 1993). Disease can be an important factor in modulating many of the processes that drive wildlife population dynamics. It can directly affect survival and reproductive success, and can also be a major influence in the specification of annual variation in demographic rates. Perhaps more subtly, disease can impact growth dynamics by altering the genetic, social, and age structures of populations.

When disease has been considered in a PVA context, traditional techniques have adopted a rather simple approach to its dynamics and its effects on the persistence of infected wildlife populations. Specifically, disease events are defined solely in terms of their impact on population demographic parameters. For example, if a disease outbreak is assumed to occur episodically with extreme consequences for the population, an age-specific survival and/or fecundity function can be developed that includes an infrequent but dramatic reduction in the parameter value. In the years between these "catastrophic" events, disease is deemed to be absent from the population. This type of approach has been used many times by the IUCN's Conservation Breeding Specialist Group (CBSG) in their implementation of PVA known as Population and Habitat Viability Assessment (e.g., Pucek et al. 1996; Werikhe et al. 1998; Jennings et al. 2001). In contrast to this "all or nothing" approach to infectious disease biology, some explicit consideration of infectious disease epidemiology can lead to more realistic simulation of disease within a given PVA. External epidemiological models can generate predictions for cyclical or other temporal patterns of disease (Grenfell and Dobson 1995). With this type of information, a PVA practitioner can then develop more sophisticated functions that model the consequent temporal trends in population demographic rates. These trends might be linear in response to increasing disease prevalence, cyclical, or follow some other specified time course.

Just as PVA models benefit from an individual-based approach, so too can simulation models of wildlife disease epidemiology gain in value from simulating the transmission dynamics of infectious disease at the level of the individual. In the presence of intrinsically unpredictable demography, the

course of an infectious disease is itself unpredictable and must be modeled as such. A review of wildlife disease models by Barlow (1995) reveals that, while there may be some elements of stochasticity included in their construction (mostly in the form of spatial heterogeneity), nearly all are population-level models that are not suitable for simulating the subtle and often non-linear demographic processes characteristic of small populations of wildlife. Based on such a review, there clearly exists a need to expand our application of classical disease epidemiology models to incorporate an individual component of stochasticity.

A "Metamodel" Approach to Linking Disease Epidemiology and PVA

The discussion presented in this section provides a case for arguing that 1) the development of an individual-based, stochastic model of infectious disease epidemiology would greatly enhance our understanding of infectious processes in small, threatened wildlife populations; 2) the physical linkage of this stochastic model with traditional PVA simulation modeling techniques would lead to a significant improvement in both our understanding of the role of disease in wildlife population growth dynamics and our ability to assess the risk of extinction of those populations threatened by human activity; and 3) an enhanced PVA approach incorporating a more sophisticated treatment of wildlife disease would greatly assist the wildlife conservation decision-making process. This integration of models from different and sometimes quite distinct disciplines has received considerable attention in the recent environmental conservation literature (e.g., Clark and Wallace 1998; Pickett et al. 1999). Others (e.g., Nyhus et al. 2002; Lacy and Miller 2002; Miller and Lacy 2002) have recently put this larger interdisciplinary discussion into sharper focus within the context of expanding our notion of traditional PVA techniques by encouraging collaboration between conservation biologists and a wide range of experts from other natural and social sciences.

The project proposed here could represent an outstanding example of close and successful collaboration between experts in population biology, disease epidemiology, and wildlife veterinary science. Leaders in interorganizational collaborative theory have recognized the difficulties in achieving such a successful synthesis (Trist 1983; Westley and Vredenburg 1996; Wear 1999; Westley and Miller 2002), and our work here may help to provide a blueprint for productive conservation biology practice.

7. Preliminary Data

Because of the specific nature of the proposed software development project, traditional preliminary experimental data do not exist. However, we can attempt to provide some results from selected simple PVA and wildlife disease modeling efforts to demonstrate our progress in formulating and evolving the ideas presented herein.

A. Mountain Gorilla Population and Habitat Viability Assessment Workshop: December 1997

Veterinary experts from the Mountain Gorilla Veterinary Center (Rwanda), International Gorilla Conservation Program, the Mountain Gorilla Veterinary Project, and the Center for Conservation Medicine were asked to identify infectious diseases that were either current or future threats to isolated mountain gorilla populations distributed in eastern equatorial Africa (Werikhe et al. 1998). Intense discussion over a 4-day period led to the construction of three catastrophic disease events: 1) an influenza-like disease occurring about once every 10 years with just 5% reduction in survivorship; 2) a severe, but not pandemic, viral disease occurring once every 10 years with measurable effects on both survival and adult female reproduction; and 3) an hypothetical viral disease with chronic cyclicity that targets the female reproductive system, occurring every 25 years but eliminating all reproduction when it strikes. These data were incorporated into an individual-based PVA simulation modeling package known as *VORTEX* (Miller and Lacy 1999; see next subsection) in order to evaluate the catastrophic impact of these disease events on mountain gorilla population persistence over a 100-year timeframe. The diseases

were assumed to occur independently, thereby allowing more than one event to affect a simulated population within a single year.

Table 1 below shows the results of these simulations. The analyses clearly indicate that the inclusion of influenza and the pandemic viral disease can significantly reduce mean population growth rates compared to that for a disease-free population. Moreover, the addition of the infrequent but severe reproductive disease results in a population that, on average, declines at a rate of about 1% per year. This simple treatment of disease as periodic catastrophes dramatically illustrated the impact that infectious disease can have on mountain gorilla populations. Perhaps more importantly, this modeling process forced the gorilla veterinarians to, for the first time, sit down and define quantitatively the types of infectious diseases they thought could have a serious impact on wild gorilla populations. This was an important step in their own process of conservation problem formulation and the recommendation of alternative solutions to the problem.

Table 1. *Impacts of selected disease scenarios (see text for description) on the viability of simulated mountain gorillas occupying the Virunga volcanoes region of eastern Africa. Output from VORTEX simulation model includes stochastic population growth rate (standard deviation), probability of extinction over the 100-year timeframe of the simulation, mean final population size (standard deviation), and average levels of population heterozygosity after 100 years. Data adapted from Werikhe et al. (1998).*

Scenario	r_s (SD)	P(E)	N_{100} (SD)	H_{100}
No disease	0.038 (0.023)	0.000	650 (5)	0.992
Diseases 1, 2	0.003 (0.095)	0.000	381 (187)	0.982
Diseases 1, 2, 3	-0.011 (0.122)	0.018	165 (151)	0.956

B. Modeling the Transmission of Measles among Mountain Gorillas and Trackers: September 2000

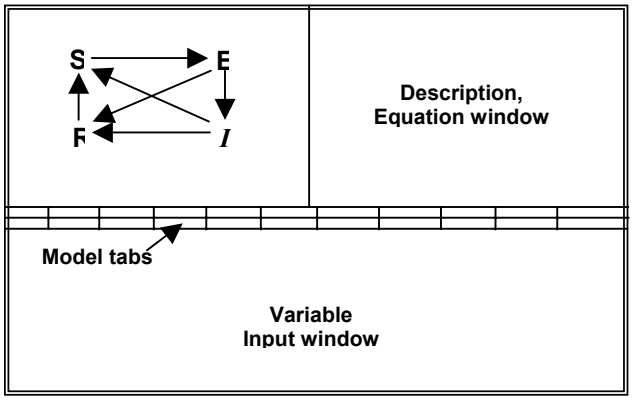
During a workshop designed to develop a set of tools optimized for wildlife disease risk assessment in conservation programs (Armstrong and Seal 2000), a group of experts led by wildlife epidemiologist Dr. Laura Hungerford (U. Maryland and collaborator with Drs. Miller and Lacy) developed a detailed model of measles transmission using the STELLA simulation environment. A graphical depiction of the model is shown in Figure 1. While preliminary, the model proved extremely valuable as a means to visualize the process, identify the critical control points, and identify relationships that may not have been immediately obvious. Moreover, with some refinement to the model algorithms and additional field data, the model could provide valuable quantitative insight into the nature of measles transmission and, therefore, assist in conservation measures designed to limit this transmission.

Figure 1. STELLA model of the transmission of measles among mountain gorillas and human trackers. See Armstrong and Seal (2000) for a detailed discussion of this model and STELLA.

8. Experimental Methods and Design

Our proposed stochastic disease model, tentatively entitled *OUTBREAK*, may be used as a separate MS Windows® modeling environment or, alternatively, incorporated as a module to the PVA modeling package *VORTEX* (see below). *OUTBREAK* would model S-E-I-R – style disease dynamics, where exposed individuals (designated E) would be tracked as well as the susceptible, infectious and recovered (resistant) individuals discussed previously. The basic conceptual algorithms of Anderson (1982) and May (1986) will be used to construct the model. The prevalence of infectious disease in a wildlife population is dependent on the number of individuals already infected, as well as on the numbers of susceptible and exposed individuals. To model infectious processes, the state of each individual in the population would be tracked, and the probabilities of transition among states would be specified as functions of the number of individuals currently in each state and of other relevant parameters such as contact rate and the latent period of infection. Multiple iterations of a given dataset would be used to generate mean population characteristics as model output for analysis.

We envision the following graphical design for *OUTBREAK*, to be built using Visual Basic:



As the user clicks on different elements of the model or, alternatively, selects one of the many corresponding model tabs, the Description window will give general information on the specific model element (disease state or transition) along with the appropriate equations that make up the mathematical treatment of that element. Moreover, the Variable Input window will show the fields (composed of drop-down boxes, radio buttons, etc.) necessary to parameterize the elements. In this way, as the user moves through the graphical depiction of the model, a complete specification of the epidemiological disease model will be complete.

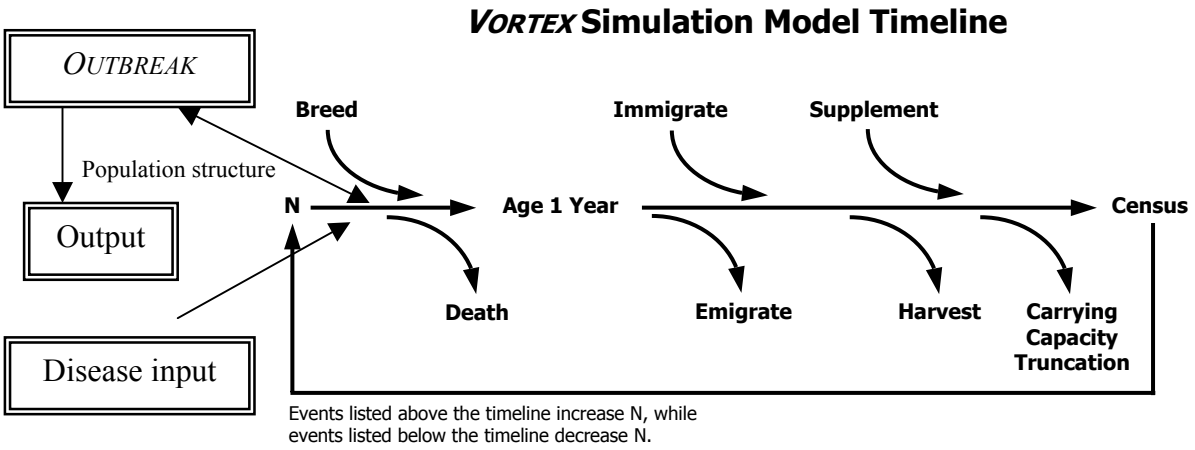
The mathematical algorithms will be programmed using Visual C++ and will run on a simulated daily time-step in order to model the details of disease transmission dynamics. In addition, relatively simple demographic information such as breeding rates and non-disease mortality for general sex-specific stages (juveniles, sub-adults, and adults) will be user-specified and used to project total population size. In addition to basic disease analysis, we intend to allow the user to include vaccination as a means of controlling disease dynamics. Model output across multiple scenarios would include real-time graphical depictions of metrics like the relative mean proportion of the population within a given disease state, mean population size, etc.

In order to test the model, we intend to develop a set of test cases using detailed epidemiological data from the literature. Preliminary searches suggest that appropriate data for natural wildlife populations are difficult to obtain, so we may modify our approach to use data from domestic livestock or perhaps even

human populations to test our product. As the basic disease processes across population types are similar, we are confident that *OUTBREAK* will have wide applicability and, therefore, can use diverse test datasets.

In addition to using *OUTBREAK* as a standalone application, the user will have the option to link the model with the popular PVA package *VORTEX* (Lacy 2000b; Miller and Lacy 1999). *VORTEX* is an individual-based stochastic simulation that requires highly specific and detailed data on a variety of demographic and other parameters of the population under consideration. Input data includes mean demographic rates for reproduction, survival, and dispersal; random variation among individuals that experience demographic events; variation in population-wide rates over time; episodic catastrophes that impact survival and/or reproduction; changes in and effects of genetic diversity; breeding systems; habitat limitations; dispersal among local populations; and managed harvest, supplementation, or translocation. Almost all demographic rates can be constant over time, can change over time, or can be specified to be functions of population density, age, sex, degree of inbreeding, other individual characteristics of the population. We are currently completing the migration of this popular software package, used around the world in both intensive research and advanced academic applications, to a flexible and powerful MS Windows® environment.

If the user intends to use *VORTEX* to investigate the projected viability of small wildlife populations impacted by disease, *OUTBREAK* will be called up to parameterize the disease of concern, and this information would then be passed on to *VORTEX* in order to allow modification of population demographic rates as a function of an individual's disease state. In this way, an individual's demographic behavior will change over the duration of the simulation as they are exposed to the infectious agent, contract the disease, and later acquire resistance (if applicable for the disease of concern).



The module should link directly to *VORTEX* immediately after the end of one time cycle, i.e., before the next cycle's breeding and subsequent mortality events (see timeline above). At this stage, we are less clear on how mortality should be handled; *OUTBREAK* could evaluate total (disease-caused and natural) mortality after receiving information from *VORTEX*, or alternatively the reverse could occur given the proper integration of data input files. Initial development will focus on this problem in order to achieve the most realistic simulation of disease-based population dynamics. In particular, we want to be able to control the rate of non-disease mortality as a function of the time of a disease event within a given year and, at the same time, the distribution of individual states during the year and at the end of the 12-month time cycle. As a result, mortality within *OUTBREAK* may well be defined on a monthly (or perhaps even daily) cycle while similar rates on *VORTEX* are defined on the standard annual cycle. As long as probabilities are defined on the appropriate timescale, with proper conversions made when necessary in

the internal code, these separate timescales of analysis will not adversely impact the integrity of the calculations.

All other aspects of population dynamics unrelated to disease – annual variation in demographic rates, catastrophic events that dramatically reduce reproduction and/or survival, dispersal and migration between metapopulations – will be handled directly within *VORTEX*. This flexibility will allow the user to develop any number of sophisticated population viability modeling scenarios with or without the additional complexity of detailed infectious disease epidemiology, and to graphically compare the results. Consequently, we will develop a versatile PVA modeling environment that is of considerably greater depth than other packages currently available. Perhaps even more importantly, we will have developed an individual-based model of wildlife disease epidemiology for broad use by the wildlife disease ecology and veterinary community.

Completion of this project will require the periodic inclusion and close coordination of a team of experienced and respected researchers in the fields of population biology, wildlife disease epidemiology, and software application programming. Collaboration will proceed largely through periodic meetings of selected group members to discuss details of product conceptualization, software development, and field testing.

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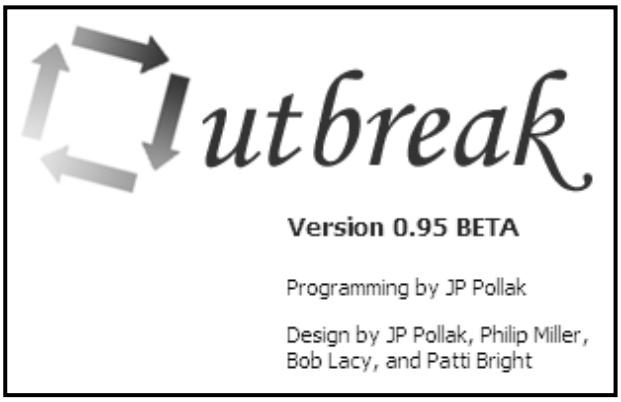
OUTBREAK
Disease Epidemiology Module
Information Worksheet



A. Population Demographic Parameters

A1. What is the window of breeding within each year?
First day of breeding (0 – 365): _____ Last day of breeding: _____

A2. At what age do individuals begin breeding? (Integer between 0 and maximum age) _____

A3. At what age do individuals stop breeding? (Integer between 0 and maximum age) _____

A4. What is the basic species life history?

	A / S	E	I	R / V	
Mortality					
0 – 1					Proportion
Subadult					
Adult male					
Adult female					
Fecundity					
% breeding					
# litters / year					Integer
Litter size					

A5. What are the initial population distributions? [Percentages or actual integers]

	A / S	E	I	R / V
0 – 1				
Subadult				
Adult male				
Adult female				

A6. What is the initial population size? (Integer greater than 0) _____

A7. What is the carrying capacity (K) of the population? (Integer greater than 0) _____

A8. How do you want to enforce the carrying capacity each year?

_____ Maintain K for each day of the year OR

_____ Apply K once per year on day X: ____

B. Population Demographic Parameters

Infectious Disease of Concern _____
(For multiple diseases to be modeled, photocopy this section as necessary)

> Note: Where appropriate, many of these parameters can be defined as more descriptive functions of, for example, age of the individual, sex, season, genotype, population density, etc.
> In addition, the user may give a mean estimate or a descriptive statistical distribution with a mean and variance.

B1. What is the primary mode of transmission? (Check all that apply)
- ○ Direct horizontal transfer between individuals of the species of concern?
- ○ Indirect horizontal transfer:
  - ☐ Through the environment?
  - ☐ Through a vector?
- ○ Vertically transmitted from:
  - ☐ Sire?
  - ☐ Dam?
- ○ Sexually transmitted?

B2. At what age (in days) does an individual become susceptible? _____
(Integer between 0 and maximum age)

B3. Of those individuals that are the appropriate age, what proportion becomes susceptible? _____
(Probability between 0 and 1)

B4. What is the average proportion of the population that is encountered each day? _____
(Proportion between 0 and 1)

B5. If encountered by an infected individual, what is the probability of transmission? _____
(Probability between 0 and 1)

B6. What is the average encounter rate per day with an outside disease source? _____
(Probability between 0 and 1)

B7. What is the incubation (latent) period of the infection (in days)? _____
(Integer between 0 and maximum age)

B8. What is the probability that an infected individual becomes infectious? _____
(Probability between 0 and 1)

B9. What is the proportion of individuals that remain chronically infectious? _____
(Probability between 0 and 1)

B10. What is the minimum amount of time (in days) an individual will remain infectious? _____
(Integer between 0 and maximum age)

B11. What is the maximum amount of time (in days) an individual will remain infectious? _____
(Integer between 0 and maximum age)

B12. After reaching the minimum amount of time for being infectious, what is the probability of recovering and becoming resistant? _____
(Probability between 0 and 1)

B13. After reaching the minimum amount of time for being infectious, what is the probability of returning to the susceptible state? _____
(Probability between 0 and 1)

B14. What proportion of the individuals that recover acquire permanent immunity? _____
(Proportion between 0 and 1)

B15. For those that do not acquire permanent immunity, how long (in days) do they remain resistant?
(Integer between 0 and maximum age) _____

C. Disease Management Options

C1. If animals are to be added to the population by supplementation, what percentage of the supplemented individuals are:

Susceptible animals? _____

Exposed animals, but not yet infectious? _____

Infectious animals? _____

Immune animals? _____

C2. Do you vaccinate your population for protection against this disease? _____

If Yes:

Vaccinate at a specified time interval (in days) _____

OR

Vaccinate when minimum disease prevalence is reached (Proportion) _____

C3. What proportion of the population do you vaccinate from each age class?

Newborn _____

Subadult _____

Adult _____

(Proportion between 0 and 1)

C4. What is the efficacy of the vaccine? (Proportion between 0 and 1) _____

C5. For how many days does the vaccine remain effective? (Integer; 0 if permanent) _____

D. Glossary

Carrying Capacity – The equilibrium number of individuals of a species that an area or defined habitat can support.

Contact Rate – The average frequency per unit time with which infected individuals contact, or otherwise put themselves in a position to transmit an infection to, susceptible individuals.

Disease – The debilitating effects of infection by a parasite; sometimes incorrectly used to refer to the disease-causing parasite. It is possible for a host to be infected by a parasite but show no symptoms of the disease.

Efficacy – An index of potency of a drug or treatment, usually estimated as the average proportion of parasites in any host killed by a single dose or short-term course of the treatment.

Endemic – A parasite whose prevalence does not exhibit wide fluctuations through time in a defined location.

Epidemic – A sudden, rapid spread or increase in the prevalence or intensity of a parasite or disease. An epidemic is often the result of a change in circumstances which favor pathogen transmission such as a rapid increase in host population density, or the introduction of a new parasite (or genetic strain of a parasite) to a previously unexposed host population.

Epizootic – The sudden spread of a parasite or disease through a non-human population; equivalent to an epidemic in human populations.

Fecundity – The capacity of a population to produce offspring; also used to describe quantitative measures of per capita reproductive rate.

Immunity – The ability to combat infection or disease due to the presence of antibodies or activated cells.

Incubation Period – The time that elapses between infection with a parasite and the onset of disease.

Infection – The presence of a parasite within a host, where it may or may not cause disease.

Infectious Disease – Disease caused by infection with a parasite which can be transmitted from one individual to another, either directly (e.g., measles) or indirectly, by a vector (e.g., malaria).

Latent Period – The period when an individual is infected but not yet capable of transmitting the infection.

Mortality – The death rate in a population.

Pandemic – A widely distributed epidemic.

Panzootic – A widely distributed epizootic, often affecting more than one host species.

Parasite – An organism exhibiting a varying but obligatory dependence on another organism, it host, which is detrimental to the survival and/or fecundity of the host.

Prevalence – The proportion of the host population with infection or disease, often expressed as a percentage. A measure of how widespread an infection or disease is.

Resistance – The ability to resist infection by a parasite.

Transmission – The process by which a parasite passes from a source of infection to a new host.

Vaccine – A sterile liquid medium containing avirulent strains of a specific parasite and often an adjuvant, introduced into the body of a susceptible individual to stimulate the production of antibodies and thus induce active artificial immunity against that parasite. May contain live attenuated parasite strains or killed parasites (or parts thereof).

Outbreak Program Flow

April 7, 2003

Following are the actions carried out in each yearly cycle of the Outbreak disease model. Prior to this yearly cycle, demographic and disease parameters are supplied and the population's initial composition is defined. If the spatial component is switched on, initial locations are chosen at random.

Begin New Year

Prep Breeding – decide who will breed on what day(s) during the year.

Simulate Each Day:

Mortality

Dispersal (if spatial component is switched on)

Breeding – breed any individuals that are scheduled to breed on a given day

For Each Individual:

Check Current Disease State and determine course of action:

If **P**: Determine on first time step if an individual will never become susceptible. If it can become susceptible, there is a range from which the day to move to S will be chosen at random.

If **S**: Check if encounters a diseased individual, and if so, does it transition to E. Then check if encounters an outside disease source, and if so, does it transition to E. If the spatial component is switched on, this is determined by proximity of infected individuals. If not using the spatial component, this is done randomly following probabilities specified by the user.

If **E**: Individual has a range of days from which it will be chosen to become infectious and transition to I.

If **I**: Determine if individual will either (1) remain permanently infectious (remain in I), (2) recover without immunity (move to S), (3) recover with immunity (move to R), or (4) die. There is a range of days during which one of the latter three will occur.

If **R**: If the individual does not have permanent resistance, there is a range from which a day will be chosen at random for the individual to become susceptible again (move to S).

Apply Carrying Capacity – if K is to be maintained each day, this is carried out each day. If to be enforced once per year, it is carried out only on that designated day.

Calculate Current Disease Prevalence

Apply Vaccinations (if selected)

Incorporating Epidemiological Models of Disease into Models of Wildlife Population Viability Using *VORTEX*[1]

Philip S. Miller, Conservation Breeding Specialist Group
Robert C. Lacy, Department of Conservation Biology, Brookfield Zoo
Jonathan D. Ballou, National Zoological Park / Smithsonian Institution

An Introduction to Population Viability Analysis

Under almost any set of circumstances, wildlife populations will fluctuate in size over time (Figure 1). These fluctuations result from random variation acting on a set of processes that, acting together, determine the dynamics of population growth. Numbers of individuals comprising a given population are determined largely by specified rates of reproduction, survival, and dispersal in addition to the ecological limitations of habitat carrying capacity. Variation in these rates is influenced by processes both intrinsic (demographic stochasticity, genetic drift and/or inbreeding depression, or deviations in age or social structure) and extrinsic (environmental variation and catastrophic events) to the population (Shaffer 1981).

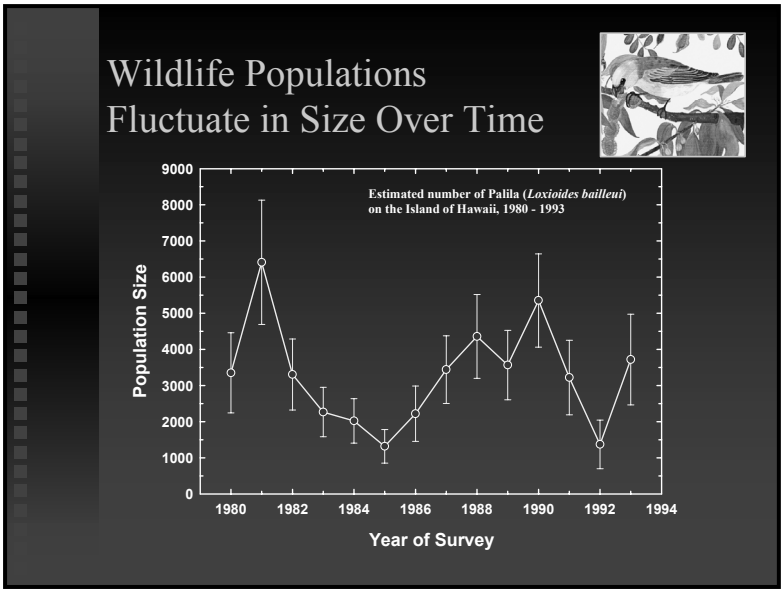


Figure 1. Census data showing annual fluctuations in estimated population size for Hawaii's palila, *Loxioides bailleui*. Figure adapted from (Ellis, S., et.al. 1992.).

Disease can be an important force in modulating many of the processes that drive wildlife population dynamics. Diseases can directly survival and reproductive success, and they can also be a major influence in the specification of annual variation in demographic rates. Perhaps more

[1] Revised and updated based on Lacy, R.C. 2000. Integrating considerations of disease into population viability analysis with *VORTEX*, in *Disease Risk Workshop Final Report* (D. Armstrong and U.S. Seal, editors). Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN).

subtly, disease can influence growth dynamics by altering the genetic, social, and age structures of populations.

While random fluctuations in size are a normal part of wildlife population dynamics, reductions in mean population size brought about by human activities can result in a greatly increased risk of extinction through the action of stochastic variation in demographic rates. This synergistic interaction between population size and stochastic extinction risk is summarized in the "extinction vortex" concept of Gilpin and Soulé (1986). Population Viability Analysis (PVA) is a technique for applying the extinction vortex concept by examining the threats to persistence of wildlife populations (Boyce 1992; Lacy 1993/4; Groom and Pascual 1998). PVA starts with a model of the forces that drive population change and then assesses population performance under a specified set of conditions (Figure 2). PVA can use empirical, analytical, or simulation methods, but most PVAs rely on simulation to assess the interacting affects of a large number of complex processes. The primary use of PVA is to estimate the probability of extinction of a population, the mean time to extinction, or other measures of population performance such as growth rate, stability, or genetic diversity. A comparison of such measures of population viability for a variety of different scenarios then allows analysis of which threats are most important. In addition, management alternatives can be compared to determine the most effective conservation strategies.

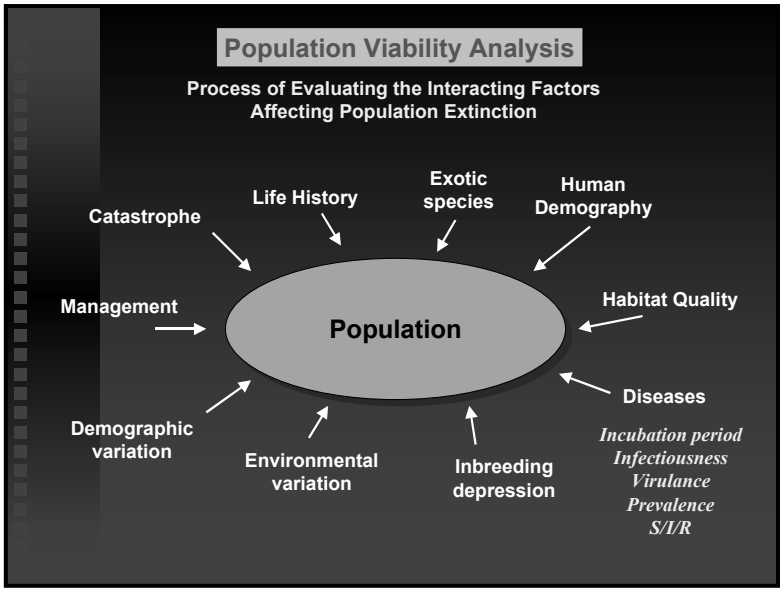


Figure 2. Generalized diagram of the forces shaping population dynamics and their inclusion in population viability analysis.

One widely used PVA model is *VORTEX* (Miller and Lacy 1999; Lacy 2000)[2]. *VORTEX* is an individual-based simulation, which requires highly specific and detailed data on a variety of demographic and other population parameters. It considers mean demographic rates for reproduction, survival, and dispersal; random variation among individuals that experience demographic events; variation in population-wide rates over time; episodic catastrophes that

[2] *VORTEX* is available from CBSG (http://www.cbsg.org ; office@cbsg.org) or from R.C. Lacy (http://www2.netcom.com/~rlacy)

impact survival and/or reproduction; changes in and effects of genetic diversity; breeding systems; habitat limitations; dispersal among local populations; and managed harvest, supplementation, or translocation. Almost all rates in *VORTEX* can be constant over time, can change over time, or can be specified to be functions of population density, age, sex, inbreeding, or other characteristics of individuals or the population.

Modeling Disease in *VORTEX*

Before we discuss the mechanisms by which the effects of disease on population viability can be incorporated into population viability analysis, a brief digression on the general nature of disease modeling in PVA is warranted. In general, opinions differ widely on how disease is to be considered in models of wildlife population viability – or whether it is to be handled at all. For example, in a recent workshop on mountain gorilla population viability and conservation (Werihke et al. 1998), wildlife veterinarians predicted that the remnant populations may be subjected to several kinds of disease: an influenza-like disease that occurs in 10% of the years and causes 5% mortality; a severe viral disease that has a frequency of 10% and causes 25% mortality and a 20% reduction in breeding for the year; and a cyclic viral disease of the reproductive system that has a frequency of 4% and causes 25% mortality and total breeding failure. The PVA showed that the hypothesized diseases would substantially threaten the long-term prospects for gorilla population persistence. As a consequence of this finding, recommended conservation actions included measures to reduce the probability of disease spreading from ecotourists to the gorillas, and increased surveillance for disease.

In contrast, a PVA workshop on the Florida panther (Seal and Lacy 1989) represents the opposite extreme (but perhaps a more typical case) in how disease can be considered in wildlife risk assessment processes. Workshop participants reached a consensus that "Disease epidemics are possible, … but we have no data that would allow estimation of the probability. … Thus, we have omitted any consideration [of disease] … from our modeling." However, the omission of disease from consideration was further justified by: "It is unlikely that the subspecies would survive a catastrophe that caused substantial mortality." It is clear that including processes that are only partially understood and/or quantified will lead to a less precise prediction of future population performance. By the same token, their inclusion into models of the extinction process can help to foster a better understanding of the population data in hand. Perhaps more importantly, comparative simulation modeling of alternative scenarios can be a valuable tool to help biologists make better population management decisions in the face of uncertain knowledge and limited resources. PVA practitioners are faced with choosing how to use the available tools on a case-by-case basis.

The effects of diseases on population viability can be integrated into the *Vortex* PVA modeling system in a variety of ways and at various levels. Disease can be modeled as a static effect on demographic rates, as a cause of variation in rates (including episodic catastrophes), as a cause of trends in rates over time, as a dynamic process in which the impacts are functions of population or individual characteristics, or as an infectious process in which the probability of an individual becoming diseased is a function of the number of other diseased individuals.

Disease as a static effect on population dynamics

When considered simply as a static effect in the PVA model, disease mortality may be one component of the mean "natural" or "baseline" mortality. Similarly, disease may be one determinant of the baseline reproductive rates (e.g., disease can be one cause of breeding failure). Disease may also be a mechanism of inbreeding depression (e.g., if inbred individuals are more likely to die of disease), or of density dependent breeding or survival. Incorporation of the impacts of disease into a PVA model as a static effect does not require that disease be identified as a cause of the natural rates. But it does require that the "baseline" rates used in a PVA model are estimated under conditions that are likely to prevail into the future, and assumes that there will be constant risks of and effects of disease. Consideration of disease as a static effect in a PVA model may be appropriate for endemic diseases that are always present as a risk in the population.

Disease as a source of variation in demographic rates

Disease that is episodic over time can be incorporated into PVA models as a contributing cause of either random variation in demographic rates over time (environmental variation) or periodic catastrophes in which survival or reproduction are temporarily impacted. For example, Figure 3 shows an example of a simulation produced by *Vortex* for a population that normally has a high potential growth rate (due to high reproduction and low mortality), but which is subjected to catastrophes that occur randomly in 2% of the years and cause 25% mortality. To analyze the effects of a disease causing such a pattern, the simulation would be repeated 100s of times, and the mean result and range of results tallied.

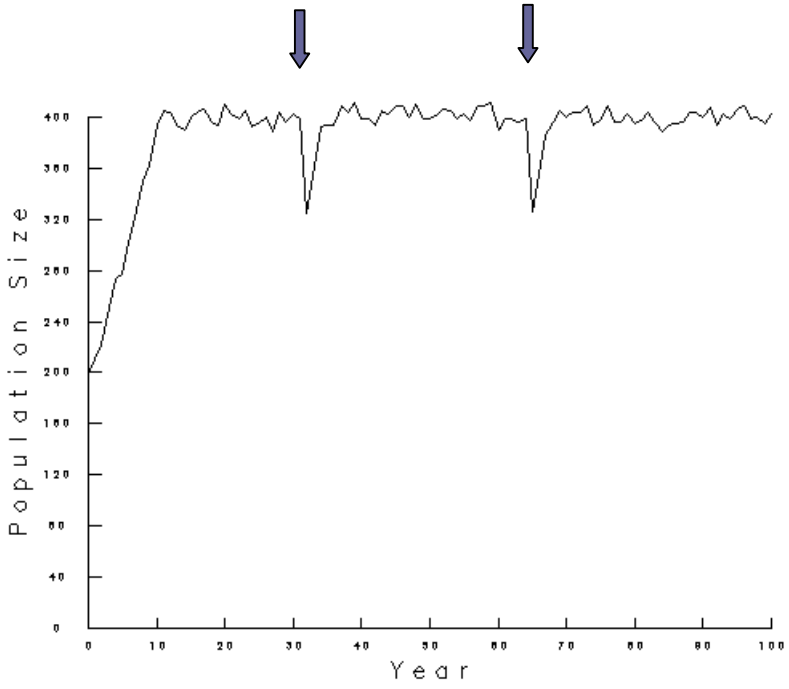


Figure 3. A simulated population subjected to a disease epidemic with a 2% annual probability of occurrence that causes an additional 25% mortality across all age classes. Arrows indicate incidence of epidemics.

Within *VORTEX*, the probability of and impacts of a disease catastrophe can be specified to be a function of population characteristics. As an example, Figure 4 shows the results of a simulation in which the effects of a catastrophe on survival are a function of population density: survival drops steeply when the population size approaches the ecological carrying capacity of the habitat.

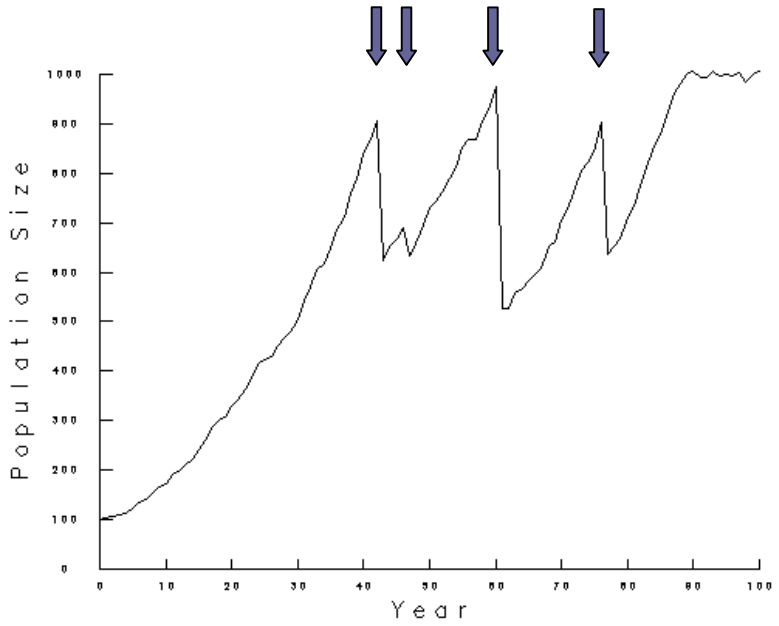


Figure 4. A simulated population subjected to a disease epidemic in which individual survival is a function of population density. Arrows indicate incidence of epidemics.

Disease as a driver of temporal trends

Epidemiological models can generate predictions for cyclical or other temporal patterns for disease (Grenfell and Dobson 1995; Scott and Duncan 1998). With this type of information at the user's disposal, *VORTEX* can model the consequent temporal trends in demographic rates. The trends might be linear (due to increasing disease prevalence), cyclical, or follow some other specified time course. Figure 5 shows a trajectory for a simulated population that is impacted by a disease that occurs are regular 10-year intervals and reduces survival by an additional 20% over "baseline" values.

Incorporating a temporal pattern of disease into a PVA requires prior development of a model of the dynamics of the disease. The time series or pattern of disease outbreaks generated by the epidemiological model then must be used to specify the temporal trend in affected demographic rates. This approach would be appropriate for modeling the impacts on population viability of a disease that follows a known and regular time course. For example, outbreaks of smallpox caused a 5-year cycle in mortality in rural England from 1557 to 1812, and whooping cough mortality in London showed a 3-year cycle with increasing amplitude from 1700 to 1812 (Scott and Duncan 1998).

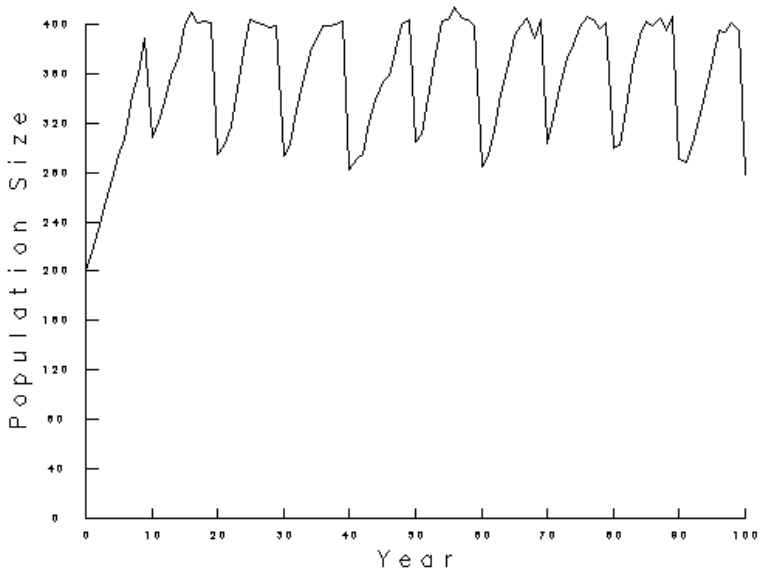


Figure 5. A simulated population trajectory in which disease epidemics occur at 10-year intervals.

Disease as an infectious process

The prevalence of infectious disease is obviously dependent on the number of already infected individuals, as well as on the numbers of susceptible and resistant individuals. To model infectious processes, the state (e.g., susceptible, latent infection, active infection, recovered, or resistant) of each individual would be tracked, and the probabilities of transition among states would be specified as functions of the numbers of individuals currently in each state (Figure 6). Transition probabilities may also be dependent on other individual characteristics, such as sex, age, inbreeding or specific genotypes. The demographic rates would then be specified to be functions of the state of the individual. For example, infected individuals may suffer 50% higher mortality or depressed breeding rates.

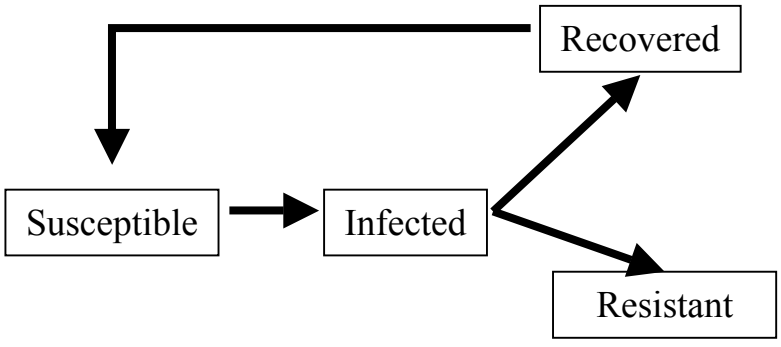


Figure 6. The S-I-R-R model of disease epidemiology. Individuals move from one state to another over time with defined probabilities. Resistant individuals are those who are no longer susceptible to re-infection. Recovered individuals are no longer infected, but can be re-infected.

In order to incorporate an infectious disease process into methods for population viability analysis, there must first be developed a model of disease transmission and recovery. The likelihood of transmission under various conditions must be known (or estimated), as well as the likelihood of recovery and the development of resistance. Unlike the simpler methods of modeling disease in PVA described above, infectious processes cannot yet be incorporated into *VORTEX* simulations (as of version 8). Further modifications of the *VORTEX* program could provide such modeling capabilities.

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Conservation Breeding Specialist Group

Species Survival Commission
IUCN -- The World Conservation Union

U.S. Seal, CBSG Chairman

Input Data Required for VORTEX

1) **Do you want to incorporate inbreeding depression?** Yes or No _____
Yes, if you think inbreeding might cause a reduction in fertility or survival
No, if you think inbreeding would not cause any negative impact

If you answered "Yes" to Question 1), then we need to specify the severity of the impacts of inbreeding by answering the following two questions:

1A) **How many lethal equivalents exist in your population?** _____
"Lethal equivalents" is a measure of the severity of effects of inbreeding on juvenile survival. The median value reported by Ralls et al. (1988) for 40 mammal populations was 3.14. The range for mammals reported in the literature is from 0.0 (no effect of inbreeding on survival) to about 15 (most inbred progeny die).

1B) **What proportion of the total lethal equivalents is due to recessive lethal alleles?** _____
This question relates to how easily natural selection would remove deleterious genes if inbreeding persisted for many generations (and the population did not become extinct). In other words, how well does the population adapt to inbreeding? The question is really asking this: what fraction of the genes responsible for inbreeding depression would be removed by selection over many generations? Unfortunately, little data exist for mammals regarding this question; data on fruit flies and rodents, however, suggest that about 50% of the total suite of inbreeding effects are, on average, due to lethal alleles.

2) **Do you want environmental variation in reproduction to be correlated with environmental variation in survival?** Yes or No _____
Answering "Yes" would indicate that good years for breeding are also good years for survival, and bad years for breeding are also bad years for survival. "No" would indicate that annual fluctuations in breeding and survival are independent.

3) **Breeding system**: Monogamous or Polygynous? _____

4) **At what age do females begin breeding?** _____
5) **At what age do males begin breeding?** _____
For each sex, we need to specify the age at which the typical animal produces its first litter. The age at which they "begin breeding" refers to their age when the offspring are actually born, and not when the parents mate.

6) **Maximum breeding age? _____**
When do they become reproductively senescent? VORTEX will allow them to breed (if they happen to live this long) up to this maximum age.

7) **What is the sex ratio of offspring at birth? _____**
What proportion of the year's offspring are males?

8) **What is the maximum litter size? _____**

9) **In the average year, what proportion of adult females produce a litter? _____**

10) **How much does the proportion of females that breed vary across years? _____**
Ideally, we need this value specified as a standard deviation (SD) of the proportion breeding. If long-term quantitative data are lacking, we can estimate this variation in several ways. At the simplest intuitive level, in about 67% of the years the proportion of adult females breeding would fall within 1 SD of the mean, so (mean value) + SD might represent the breeding rate in a typically "good" year, and (mean value) – SD might be the breeding rate in a typically "bad" year.

11) **Of litters that are born in a given year, what percentage have litters of …**
1 offspring? _____
2 offspring? _____
3 offspring? _____
4 offspring? _____
(and so on to the maximum litter size).

12) **What is the percent survival of females …**
from birth to 1 year of age? _____
from age 1 to age 2? _____
from age 2 to age 3? _____ (no need to answer this if they begin breeding at age 2)
from age *x* to age *x*+1, for adults? _____

13) **What is the percent survival of males …**
from birth to 1 year of age? _____
from age 1 to age 2? _____
from age 2 to age 3? _____ (no need to answer this if they begin breeding at age 2)
from age *x* to age *x*+1, for adults? _____

14) **For each of the survival rates listed above, enter the variation across years as a standard deviation:**
For females, what is the standard deviation in the survival rate
from birth to 1 year of age? _____
from age 1 to age 2? _____
from age 2 to age 3? _____ (no need to answer this if they begin breeding at age 2)
from age *x* to age *x*+1, for adults? _____

For males, what is the standard deviation in the survival rated
from birth to 1 year of age? _____
from age 1 to age 2? _____
from age 2 to age 3? _____ (no need to answer this if they begin breeding at age 2)
from age *x* to age *x*+1, for adults? _____

15) **How many types of catastrophes should be included in the models? _____**
You can model disease epidemics, or any other type of disaster, which might kill many individuals or cause major breeding failure in sporadic years.

16) For each type of catastrophe considered in Question 15),
What is the probability of occurrence? _____
(i.e., how often does the catastrophe occur in a given time period, say, 100 years?)
What is the reproductive rate in a catastrophe year relative to reproduction in normal years? _____
(i.e., 1.00 = no reduction in breeding; 0.75 = 25% reduction; 0.00 = no breeding)
What is the survival rate in a catastrophe year relative to survival in normal years? _____
(i.e., 1.00 = no reduction in breeding; 0.75 = 25% reduction; 0.00 = no breeding)

17) **Are all adult males in the "pool" of potential breeders each year?** Yes or No _____
(Are there some males that are excluded from the group of available breeders because they are socially prevented from holding territories, are sterile, or otherwise prevented from having access to mates?)

18) If you answered "No" to Question 17), then answer at least one of the following:
What percentage of adult males is available for breeding each year? _____
or
What percentage of adult males typically sires a litter each year? _____
or
How many litters are sired by the average breeding male (of those that sired at least one litter)? _____

19) **What is the current population size? _____**
(We will assume that the population starts at a "stable age distribution", rather than specifying ages of individual animals in the current population.)

20) **What is the habitat carrying capacity? _____**
(How many animals could be supported in the existing habitat?)
(We will assume that the habitat is not fluctuating randomly in quality over time.)

21) **Will habitat be lost or gained over time?** Yes or No _____
If you answered Yes to Question 21), then …

22) **Over how many years will habitat be lost or gained? _____**

23) **What percentage of habitat will be lost or gained each year? _____**

24) **Will animals be removed from the wild population (to bolster captive stocks or for other reasons)?** Yes or No _____
If "Yes", then,
At what annual interval? _____
For how many years? _____
How many female juveniles? _____ 1-2 year old females? _____ 2-3 year old females? _____ adult females? _____ will be removed each time.
How many male juveniles? _____ 1-2 year old males? _____ 2-3 year old males? _____ adult males? _____ will be removed each time.

25) **Will animals be added to the population (from captive stocks, etc.)?** Yes or No _____
If "Yes", then,
At what annual interval? _____
For how many years? _____
How many female juveniles? _____ 1-2 year old females? _____ 2-3 year old females? _____ adult females? _____ will be added each time.
How many male juveniles? _____ 1-2 year old males? _____ 2-3 year old males? _____ adult males? _____ will be added each time

Note: VORTEX has the capability to model even more complex demographic rates, if a user thinks that greater specificity is needed. For example, breeding or survival rates could be specified as functions of age. Contact Philip Miller, Program Officer with CBSG if you would like to learn more about this additional flexibility.

ANIMAL MOVEMENTS AND DISEASE RISK

A WORKBOOK

5TH EDITION

Costa Rica

28-30 April 2003

10. DECISION ANALYSIS MODELING SOFTWARE AND EXAMPLES

Zoo Risk Assessment meeting, June 28-30, 2001

Barbara Corso, DVM, MS
USDA/APHIS/VS/Centers for Epidemiology and Animal Health
Ft Collins, CO

Topics
1.) @Risk software, used for risk assessments
2.) Spatial Analysis projects, including vector mapping

@Risk
Software distributed by Palisades Corporation. Add on to a spreadsheet, with different versions for different spreadsheet programs. We use Excel, with the appropriate version of @Risk.

Start with the workbook we have used here. When you get to the point where you are doing a quantitative assessment using a spreadsheet program, @Risk is helpful because it allows you to enter input values as distributions, rather than point estimates. Many different distributions are available, including many that are fairly standard for certain circumstances.

Entering distributions allows you to account for variability of a factor within a population, or for uncertainty in our knowledge about the level of that factor. @Risk will run hundreds or thousands of iterations, using values from within the distributions that were selected as inputs. It will calculate the probability of the outcome(s) of interest, producing probability curves rather than a single value for the final risk. Sensitivity analysis and other outputs are also available. And you can change the input values to account for mitigation efforts - vaccination, testing, etc. - and recalculate to see what effect that activity has on the output distribution.

Be aware that explanation and presentation of results can be difficult for some audiences to grasp. Sometime people want a single number. We usually present results in the format of: '95% of the simulations resulted in less than one introduction of disease in 10,000 years'.

Various types of @Risk projects we have been involved in:
1.) Import trade risk assessments: Assess risk of importing agents through importation of various commodities. For example, assess the risk of introducing Hog Cholera (Classical Swine Fever) into the US through importation of swine semen from the European Union.

2.) Introduction of agents through other means: Assess the probability of agent spread given certain management factors, e.g., risk of introducing hog cholera into the US swine industry by feeding food waste to swine

3.) Spread of an agent: Assess the probability of agent spread to new population, e.g., likelihood of spread of TB from deer to cattle in Michigan, or the likelihood of introduction of rabies into Hawai'i under new protocol.

Spatial Analysis

There are a couple of questions that come up frequently when we are asked to do spatial analysis projects. Can we relate cases to environment, and then use that information to assess risk or predict future problems? And can we look at location, distance and direction, analyze what we see, and determine whether proximity to something (like a case, or a slaughter plant, or a swamp) affects probability of infection.

Some data needed for spatial analyses of these types:

Information about location and number of cases,

Spatial base information: Data are available from different sources - satellite images; thematic layers showing environmental factors (such as elevation, precipitation, vegetation, slope), roads and waterways, and many other spatial base layers. Others will be important landmarks specific to a particular issue, and will likely need to be added, such as slaughter plants or feeding grounds.

There are a number of analytical tools to help you make sense of the data – statistical packages, modeling programs, techniques like cluster analysis. Use those tools to see whether the location or distribution of cases is related to environmental factors or spatially related to other landmarks.

Specific spatial analysis projects we are involved in:

1.) Vector mapping: Development of a database describing the geographic distribution of ticks in the US. This is focused on ticks that affect livestock, poultry and wildlife. Information is being collected from many sources. Once that is complete, we will look at environmental factors - elevation, vegetation, precipitation, slope - to see if presence is related to environmental factors. It is anticipated that this effort will assist in the future when ticks or tick-borne diseases of concern are identified, because some of the groundwork on where vectors may be found, or may survive and thrive, will have already been done.

This effort is also related to the invasive species initiative. There is a great deal of concern about potential introduction of new vectors, or new diseases transmitted by vectors. Knowledge about what ticks are currently present, and where might exotic species gain a foothold, will be valuable for evaluations of potential consequences.

2.) Additional Tick related projects: Geographic analysis of ticks and tick-borne diseases in Morocco; Analysis of ecological factors associated with the introduction of *Boophilus* spp ticks in the Lower Rio Grande Valley of Texas

3.) Mosquitoes related projects: Ecological analysis of West Nile Virus infection in equids; Spatial and spectral habitat characterization of enzootic foci of VEE virus activity in Venezuela, Colombia and Peru

4.) Projects related to other vectors: Midges - Bluetongue in North and South Dakota and Nebraska; Flies - Vesicular Stomatitis, Equine Infectious Anemia

EXAMPLES:

MOUNTAIN GORILLA

Participants: Laura Hungerford, Patty Klein, Mike Cranfield, Genevieve Dumonceaux, Barbara Corso, Mark Atkinson, Shelley Alexander, Dominic Travis, Tom Meehan, Jim Else, Sue Brown

Step 1: Tell the story –

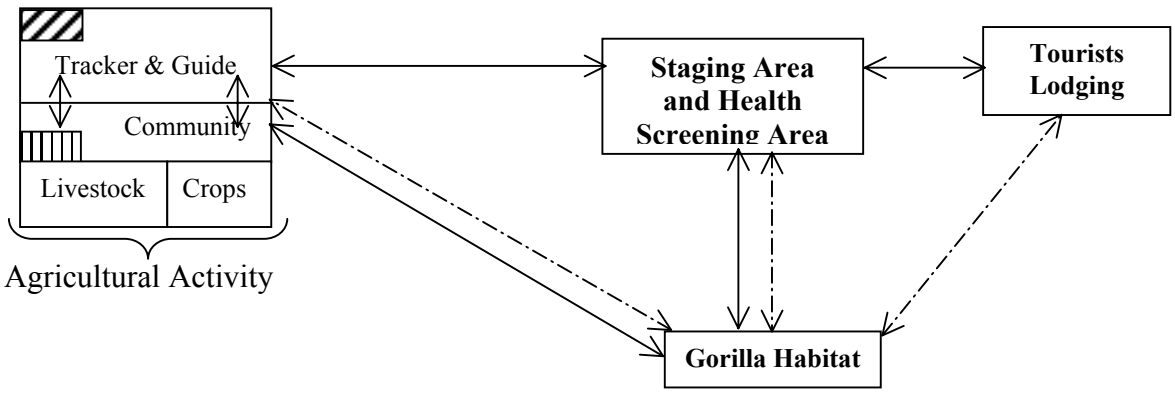
- Bwindi Park Gorillas
- Tracker & guides are the source
- Scabies originates from the local community – i.e., one of the few diseases that does NOT stem from the trackers & guides
- The diseases of most concern for the gorillas is measles (affects population for a few months) and/or tuberculosis (continually affects population for years)

Step 2: Define the question –

a) Risk of transmission of disease into the gorillas
b) What is the likelihood of introducing Scabies into habituated gorilla population?
c) What is the likelihood of introducing Cryptosporidia into habituated gorilla population?
d) What is the likelihood of introducing measles into habituated gorilla population?
e) What is the likelihood of introducing measles into habituated gorilla population?

Species of Concern: 1) Humans, 2) Gorillas, 3) Other (Habituated) Primates

Step 3: T = Tracker, G = Guide
Human movement = solid line arrows
Gorilla = dashed arrows
- Cryptosporidia Vector
- Scabies Vector



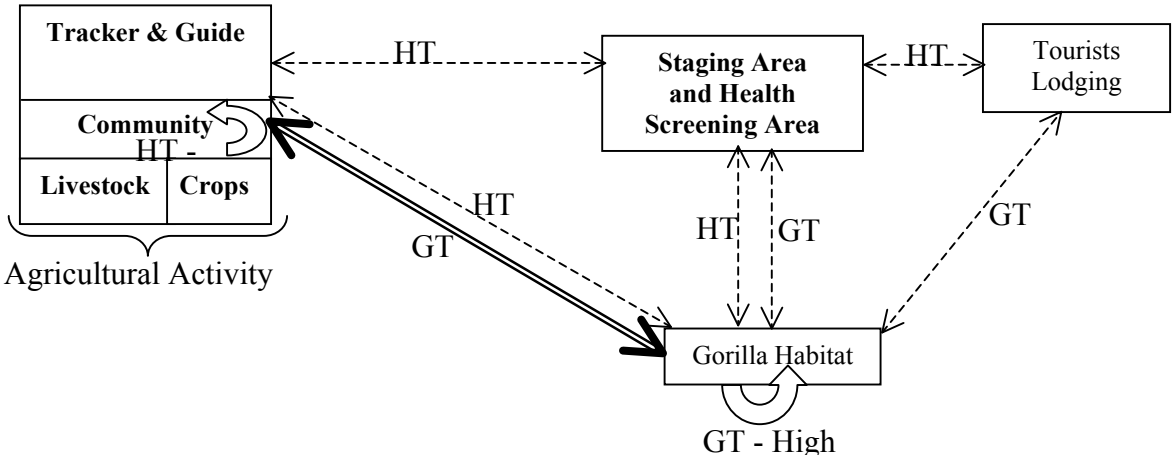
Procedures done at all points:
(a) At T & G/Community/Agricultural Activity Area – community health programs (basic), basic vet care
(b) At the Staging/Health Screening Area – educational program

Step 4 – Identify all potential hazards for **Scabies**:

Source Point	Hazard Risk Assessment
Trackers & Guides	Low
Local Community	High
Livestock/Crops	None
Staging/Health Screening Area	Low
Tourist Lodging	None
Gorilla Habitat	High

Step 4 - **Scabies** Transmission:

Low probability of transmission rate = dashed arrows
Medium probability of transmission rate = solid line arrows
High probability of transmission rate = double line arrows
HT = Human Transmission/Movement
GT = Gorilla Transmission/Movement



ASSUMPTIONS/CONCLUSIONS:

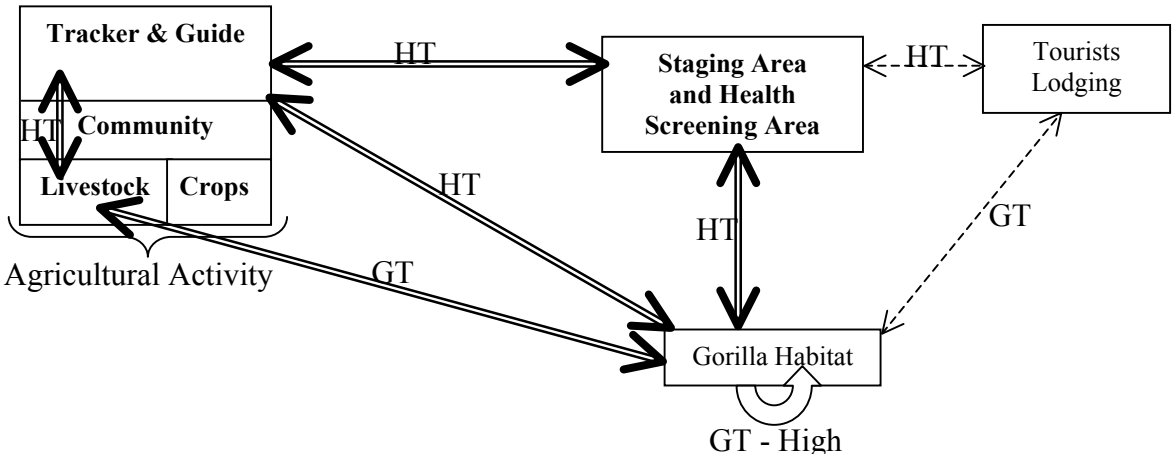
- Probability of transmission from T & G is low
- Critical Control Point (CCP) is Gorilla movement to & from community
- CCPs are within the community, Gorilla to Gorilla within habitat, and community to gorilla

Step 4 – Identify all potential hazards for **Cryptosporidia**:

Source Point	Hazard Risk Assessment
Trackers & Guides	High
Local Community	Low
Livestock/Crops	High
Staging/Health Screening Area	Low
Tourist Lodging	Low
Gorilla Habitat	High

Step 4 - **Cryptosporidia** Transmission:

Low probability of transmission rate = dashed arrows
Medium probability of transmission rate = solid line arrows
High probability of transmission rate = double line arrows
HT = Human Transmission/Movement
GT = Gorilla Transmission/Movement



ASSUMPTIONS/CONCLUSIONS:

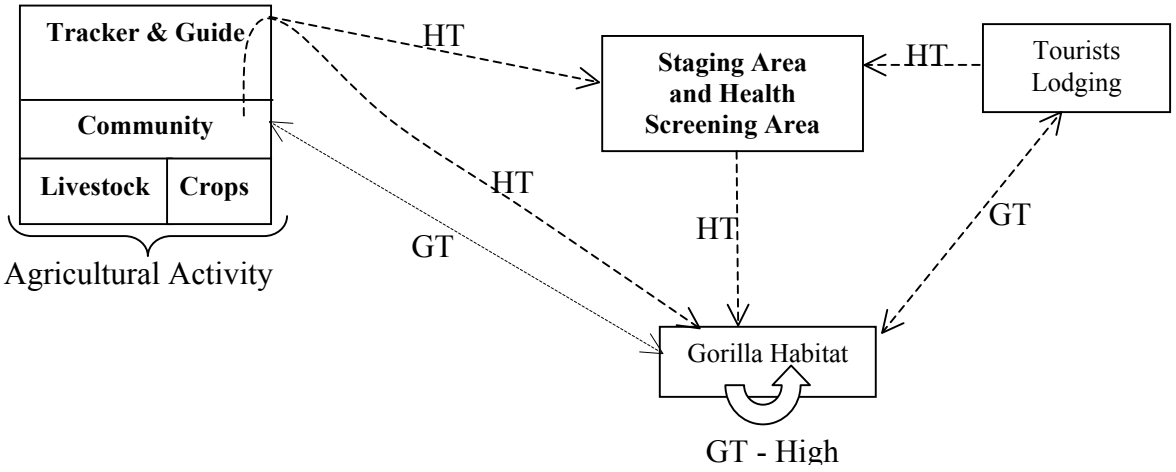
- Not critically significant
- 4 Critical Control Points (CCPs) = Gorilla to Livestock; Livestock to T&G; staging area to gorillas; and T&G to Gorilla

Step 4 – Identify all potential hazards for **Measles**:

Source Point	Hazard Risk Assessment
Trackers & Guides	Low (> 0)
Local Community	Low (> 0)
Livestock/Crops	None
Staging/Health Screening Area	Low (> 0)
Tourist Lodging	Low (> 0)
Gorilla Habitat	None

Step 4 - **Measles** Transmission:

Low probability of transmission rate = dashed arrows
Medium probability of transmission rate = solid line arrows
High probability of transmission rate = double line arrows
HT = Human Transmission/Movement
GT = Gorilla Transmission/Movement



ASSUMPTIONS/CONCLUSIONS:

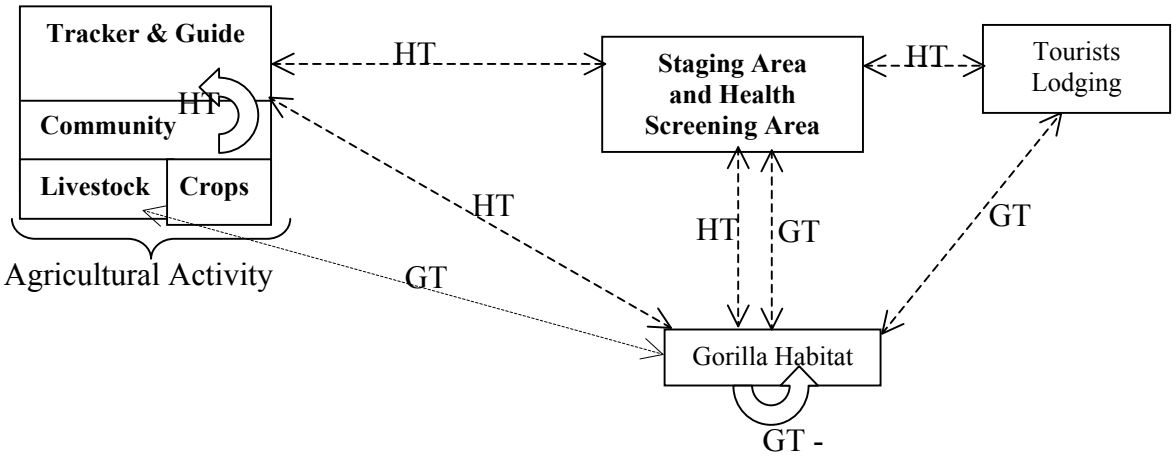
- Probability of transmission from T & G and/or tourists is extremely low but effect if it occurs is really bad
- Risk of transmission is extremely low
- CCP is within Gorilla population
- Need to modify destination population

Step 4 – Identify all potential hazards for **Tuberculosis**:

Source Point	Hazard Risk Assessment
Trackers & Guides	Medium/Moderate
Local Community	Medium/Moderate
Livestock/Crops	Low
Staging/Health Screening Area	Medium/Moderate
Tourist Lodging	Low
Gorilla Habitat	None

Step 4 - **Tuberculosis** Transmission:

Low probability of transmission rate = dashed arrows
Medium probability of transmission rate = solid line arrows
High probability of transmission rate = double line arrows
HT = Human Transmission/Movement
GT = Gorilla Transmission/Movement



ASSUMPTIONS/CONCLUSIONS:

- Extremely low risk of transmission
- No effective treatment → significant health problem and morbidity/mortality
- Critical Control Point (CCP) is within the community and gorilla to gorilla

ACTIONS:

Community CP

1. Increase community and public health programs/education
2. Employee health programs
3. Increased livestock health programs/education
4. create buffer zone

Staging Area CP

1. Tracker and guide personal hygiene
2. Tourist personal hygiene

Habitat CP

1. Vaccination program
2. Treatment

Stella Working Group Summary of Diagram

We developed this model as a working draft to allow the group to become familiar with the Stella program.

Set up:
Modeled as transmission of disease among gorillas, transmission among children of trackers, transmission among other children in the village, trackers used as route of exposure of measles to the gorillas.

Assumptions:
1. Gorilla contract measles (from humans and each other)
2. Humans act as fomites for the measles virus
3. Trackers developed immunity to measles as adults
4. Naive populations = all but trackers
5. Negligible impact of transmission tracker to tracker.
6. Closed populations
7. Random contacts
8. Random dispersal
9. Human adults that are not trackers are irrelevant (only trackers have contact with gorillas)
10. That all people infected recovered to immunity.

Identifying data:
Other kids = 5000
Trackers kids = 700
Trackers = 110
Gorilla population = 320

Noncontact gorillas = 60
Contact gorillas = 260
Vaccine programs as 98% efficacy for gorillas and people
Contact rate sick child to child of 1:10
Contact rate for trackers to gorillas in contact groups of 1:20
Contact rate for noncontact gorillas to contact gorillas of 1:2

Run and evaluate scenarios:
1. Measles goes through the population
2. Vaccinate just the trackers children
3. Vaccinate all children
4. Vaccinate gorillas only

Results of simulations:
Vaccinating the gorillas only was the most effective way to minimize the incidence of measles in the gorilla population.
Reevaluate model again, and again and again.....

Summary:

Process of developing the model:
Identification of the problems to address.
Assemble a group individuals with diverse experience and training.
Employ someone who has a clue about Stella.
Begin to draw a conceptual picture of the problems you are addressing.
Develop assumptions.
Determine control points of the model.
Input data into the model (if possible real data used and otherwise bet estimates).
Run the model.
Evaluate the data, model and graphs resulting.
Reevaluate the appropriateness of the data entered and the relationships created.
Continue to refine and improve the model (to infinity).

Question:
Does this approach provide benefit in exploring a complex problem?
Answer:
Yes, it allows you to visualize the process, identify critical control points, and identify relationships that may not have been obvious, clearer idea of information needed to acquire.

Question:
Can this approach give you a quantitative answer?
Answer:
With more refinement and enough good data it may give you quantitative answers.

Decision Tree Cost Analysis- Human ➜ Gorilla measles

Description and Interpretation

Three scenarios were assessed. The first involved an assumed prevalence in the in-contact human population of 10% and screening for the disease in these individuals is conducted by cursory inspection and observation of clinical signs only. The sensitivity of this method was assumed to be 50%. The cost was assumed to be zero.

Scenario one- physical inspection of trackers

COST?	parameter	(p)	value	comment
-	Prevalence	0.1	$0	
+	Test	0.5	$0	Cursory observation for signs of infection
-	Viability	0.01	$0	
-	transmission	0.5	$0	
TOTAL		0.0002	$0	

In the second scenario the screening test method used was a hypothetical PCR of clinical samples from every in-contact human. The sensitivity of this method was assumed to be 99%. Specificity was assumed to be 75%. Additional assumptions were that positive in-contact humans were excluded from the workforce. Based on this specificity the probability of a false positive individual is 0.225. This created the requirement for an additional 25 (rounded) individuals on the workforce and resulting labor cost increases. This was also based on a daily application of the method- may not be realistic at all. The effect of frequency of PCR testing (daily, weekly, quarterly, annually) on the sensitivity value of the method (not of the test) must be considered. The costs incurred were the test costs and the labor costs. The probability of disease (agent) introduction into the gorilla population was reduced to 0.00005 in this model.

Scenario two- PCR testing of trackers

COST?	parameter	(p)	value	comment
-	Prevalence	0.1	0	
+	Test	0.01	25 x 100 75	PCR oronasal swab Labor increase
-	Viability	0.01	0	
-	transmission	0.5	0	
TOTAL		0.00005	2575	Per test application (day?/week/quarter) Need to figure change in sensitivity due to change in testing frequency

Assumptions:

- 100 tracker/guards at $3/day

- PCR test cost = $20
- Increased sensitivity of PCR increases false + % so that (p) = 0.225 therefore workforce required increases

The third scenario implemented vaccination of the in-contact humans. Vaccine efficacy was assumed to be 99% and therefore prevalence dropped to 1%. Testing was limited to inspection for signs and therefore 50% efficacy was assumed. This approach dropped cost to a one-time investment of $2.00 per vaccinate or initial $200 outlay. The risk probability went to 0.000025.

Scenario three- vaccination of trackers

COST?	parameter	(p)	value	comment
-	Prevalence	0.01	200	Vaccine efficacy reduces prevalence to 1%
+	Test	0.5	0	Inspection for signs
-	Viability	0.01	0	
-	transmission	0.5	0	
TOTAL		0.000025	200	One time cost

Assumptions:

- Vaccine cost = $2/dose
- 100 trackers/guards vaccinated
- Vaccination reduces prevalence to 1%

Recommendations

Based on these data and models it is clearly more cost beneficial to vaccinate the in-contact humans, however the use of PCR as screening test reduces risk of measles introduction five-fold. These conclusions appear to differ from those obtained using the Stella model, however, this disparity may be due to the complexity of the Stella model, that is- the addition of temporal considerations and additional variables which may effect the outcome.

Decision Analysis III: Capillaria infestation in Whooping Cranes

What is the risk of introducing a non-North American Capillaria species into Florida from released captive birds?

All numbers used in this Decision Tree are best guesses based on the experience of the whooping crane program and input from the group members.
-It was estimated, based on WC flock history, that there is a 30% infection rate of this capillaria in release age birds.
-It was GUESSED that the fecal sedimentation test used will pick up 60% of infected birds.
-It was estimated that treatment with ivermectin and fenbendazole is 80% successful.

Definition of a False + : Bird infected with the NORTH AMERICAN species of Capillaria, not the foreign species for which we are evaluating risk.

Conclusion: There is a 0.024 (0.010 + 0.014 false negatives) probability of introduction of the non-North American capillaria when these birds are moved.

Decision Tree Cost Analysis- *Capillaria* ➜ Cranes

Description and Interpretation

The originally presented decision tree was expanded to include all possible animal treatment/test groups and their associated probabilities. Also to calculate the number of animals that are eligible for release in each scenario and associated costs.

Assumptions:

- Capture/handling costs = $610 (60 hours effort)
- Fecal sedimentation = $10/tst x 24 = $240
- Re-testing has same sensitivity and specificity as initial
- Treatment = $3/ bird x 24 = $72
- Re-treatment has same efficacy as original
- No mortality due to handling the birds

Scenario one- Test, treat all, test: $610+240+240+72		$1162 ($552)*
Release ~19	(p)= 0.024	[$29/bird]

*Capture and handling occurs annually for health screening. Therefore the figure in parentheses excludes this cost and actual cost per bird is based on this figure.

The above scenario represents the current protocol of testing and treatment. This results in approximately 19 birds eligible for release at a cost of $29.00 per bird and a probability that a released bird is Capillaria infested of 0.024 (~2:100).

The probability of false negative birds is calculated as follows. (See Decision Tree)

(p) False Negative = (0.3 x 0.4 x 0.2 x 0.4) + (0.3 x 0.6 x 0.2 x 0.4) = 0.024

The number of release candidates was calculated as follows. (See Decision Tree)

= (true negatives + false negatives)
= [(24 x (0.11+0.45+0.077+0.12)] + [24 x 0.024]

Scenario two- Treat, Test: $610+240+72		$922 ($312)
Release ~ 19	(p)= 0.024	[$16.42/bird]

Scenario two modifies the decision tree by excluding the first test requirement. This collapses the second decision node (i.e., eliminates the first test-decision point) and results in the same probability that a released bird is Capillaria infested for a lower cost. The probability is the same because no management decision is made based on the first test.

Scenario three- Test, treat+, retest+: $610+21+240+70		$941 ($331)
Release ~ 23	(p)= 0.12	[$14.39/bird]

This scenario assumes only those birds testing positive on the first test are treated and re-tested. This ends the branching of the decision tree at all negative test levels. Therefore, the result is an increase in the number of release candidates, however, the probability of false negatives increases as well. As a result the cost per release candidate is further reduced.

Scenario four- Treat only: $610+72		$682 ($72)
Release ~ 24	(p)=0.06	[$3/bird]

The treatment-only scenario simplifies decision tree analysis by eliminating all test nodes. The number of release candidates is maximized, the cost per candidate is minimized but the probability of releasing an infested bird is 2.5 times greater than scenarios one and two.

Scenario five- Treat x 2: $610+610+72+72		$1364 ($72)
Release ~ 24	(p)=0.012	[$31.41/bird]

Scenario five illustrates the effect of adding a second treatment. The cost increases because of the additional handling required but risk decreases five-fold. It should be noted that twice the handling increases health risk for the birds. The added handling cost could also be eliminated by treatment at time of release.

Concluding Comments: The costs incurred in the above scenarios should be kept in perspective of the overall cost of the program estimates of $40,000/ bird. Costs due to other disease management will be incurred as well.

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11. COST ANALYSIS SPREADSHEET

Decision Analysis III: Capillaria infestation in Whooping Cranes

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Concluding Comments: The costs incurred in the above scenarios should be kept in perspective of the overall cost of the program estimates of $40,000/ bird. Costs due to other disease management will be incurred as well.

Goal of Action: Return SCI Shrike from Zoo to SC Island

Identify Question: Concern for not introducing disease to SCI shrike population

Identify Possible Diseases (Matrix)

	Infectivity	Transmission	Disease
Avian TB	+	+	+
Chlamydiosis	+	+	+
Sarcocystis	?	?	?
Hematozoa	?	+	?
Avian Pox	+	+	+

AVIAN TUBERCULOSIS

		P	Acc P	Costs
Prevalence	Infected	0.10	0.10	
Liver Bx	AFB -	0.20	0.02	$100
AFB Cx feces	AFB -	0.90	0.02	$30
Quar 45 days off-island	Survive	0.90	0.02	$200
Isolation 30d on-island	Survive	0.95	0.02	$50
AFB Cx feces	AFB -	0.90	0.01	$30
Viability of organism in environment	Organism survives	0.80	0.01	
Transmission	Yes	0.30	0.003	
Probability of infected bird at end		**0.003**		**$410**

3/1000

CHLAMYDIOSIS

Prevalence	Infected	0.10		Prevalence	Infected	0.10
EBA	Negative	0.10	$15	Exam	No lesions	0.20
Quar 45 days off island	Survive	0.50	$200	Quar 45 d off-island	No lesions	0.20
Isolation 30d on-island	Survive	0.50	$30	Isolation 30 d on-island	No lesions	0.20
EBA	Negative	0.10	$15			
Viability of organism in environment	organism survives	0.80		Viability of organism in environment	Organism survives	0.95
Transmission	yes	0.80		Transmission	yes	0.30
Probability of infected bird at end		**0.00016**	**$260**	**Probability of infected bird at end**		**0.000228**

16/100,000

AVIAN POX

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12. MAPPING – MAPS AND GIS

MAPPING – MAPS AND GIS

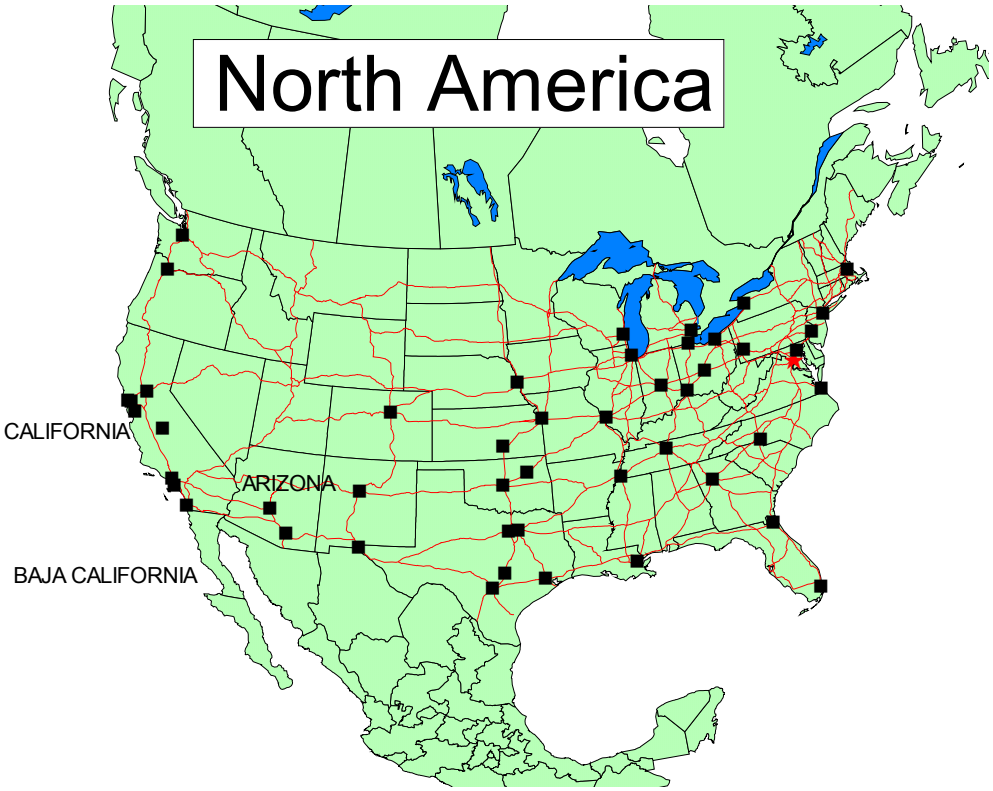
Maps are used to orient people to their geographic environment. At the most basic level, maps provide the spatial context necessary for defining problems, identifying risk, and assessing risk. The use of maps in workshops takes many forms and varies in sophistication. The simplest maps are drawn by hand on paper and represent features of the landscape useful for orientation. More detailed and accurate maps can be custom generated by computers, acquired from widely available software libraries (e.g, Microsoft Excel, Microsoft Encarta, Corel Quattro,), downloaded from the Internet (e.g., http://mapping.usgs.go), or copied from resources such as atlases. Finally, satellite images and aerial photographs can be acquired in hard copy and digital formats.

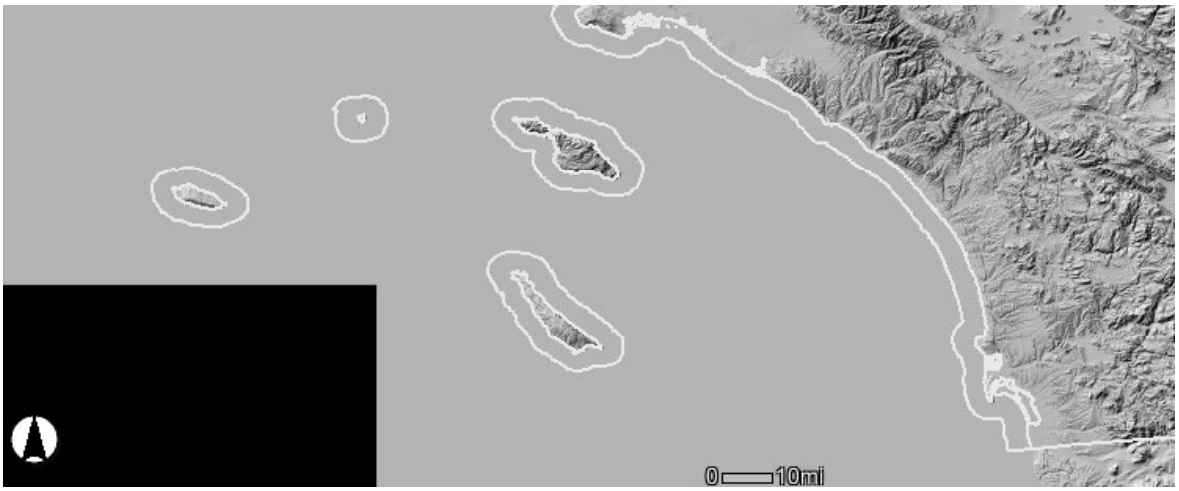
For many workshops, the most useful maps are hard copies that can be drawn on. Real-time computer mapping can be a very useful adjunct to hard copy maps, but this requires considerably more technical equipment and expertise. Finally, Geographical Information Systems (GIS) can be used to display, manipulate, and analyze spatial data represented on maps. Again, the added level of sophistication, computer hardware requirements, and expertise limit the utility of GIS for workshop applications.

In most workshop settings, a variety of maps at different resolutions and detail should be used to provide spatial context at several scales. A thoughtful pre assessment of information requirements by workshop organizers and participants allows for the acquisition of maps that are relevant to workshop topics. As an example on the following pages we provide a series of maps at different scales showing the location and features of the Channel Islands in California that might be useful for assessing disease risks of foxes that inhabit the islands. These maps were created in less than 1 hour using Microsoft Excel and a free Internet library provided by the U.S. Geological Service.

Geographic Information Systems

Shelley Alexander and Paul Paquet

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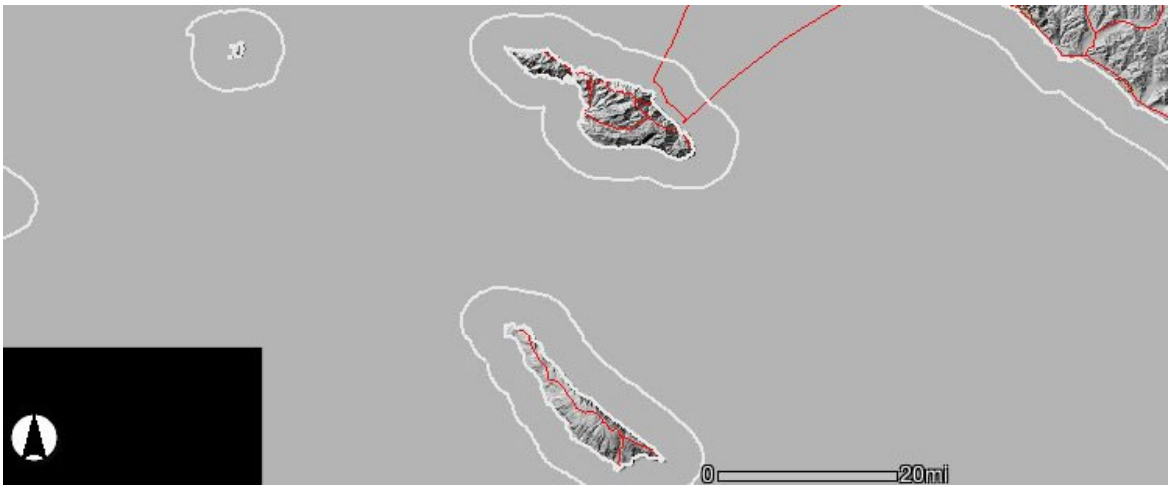
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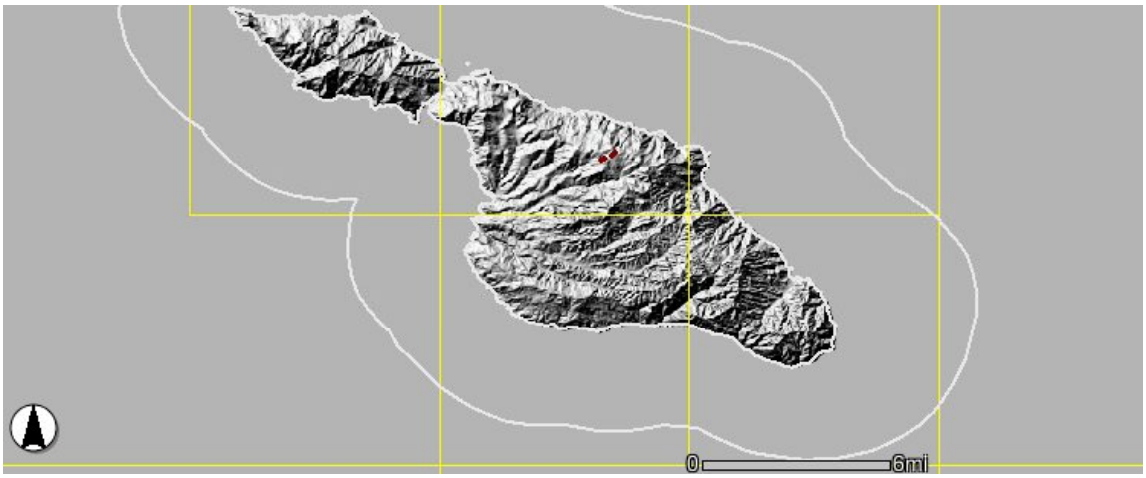
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Recent developments in *Decision Science* suggest that a much wider range of strategies can be deployed in risk analysis. A flexible procedure is the *Ordered Weighted Average* (OWA), recently introduced to GIS. This is a procedure that is somewhat related to Weighted Linear Combination, but which is capable of producing a virtually infinite variety of strategies as illustrated below.

The OWA procedure results in decision strategies that vary along two dimensions: risk and tradeoff. At one extreme, we have a solution which assumes the least risk possible and consequently allows no tradeoff (the lower left corner of this triangle).

The problem of reintroduction of species and disease transmission lends itself to analysis with Geographic Information Systems (GIS), as these are inherently spatial processes. Many advances in GIS modelling capabilities have occurred. This document briefly details the construction of a probability or risk surface for the reintroduction of a species.

New advances include the inclusion of probabilities and Bayesian inference in developing predictive surfaces. Also, modules such as Cellular Automata, may be of interest as this offers a mechanism to spatially describe the movement of a disease in the landscape. Rules of transmission may be assigned to a start site and the behavior of the disease will depend on these rules and the values of surrounding cells.

Probability surfaces can be used to assess the risk of disease for animals at different sites on the landscape. To begin, one must identify the factors that influence disease risk (i.e. trails on which guides take tourists to see Gorillas). The relationship between this metric (trails) and disease spread must be specified (See Figure 1,2, and 3). For example, does the likelihood of infection

decrease with distance to the trails. GIS allows the user to develop a surface of distance to trails. This surface can then be restated as a probability of disease transmission. The relationship can be specified in a number of ways (linear monotonically decreasing, sigmoidal monotonically increasing, etc) in the GIS. The example below simply applied a linear monotonically decreasing probability of infection, as a function of distance from any of the human factors (e.g.trails). A Bayesian modelling module combines all probability surfaces into a final risk surface (Figure 4), which may then be used to determine the associated risk for each potential reintroduction site.

The use of GIS to determine the landscape attributes that may underlay disease transmission or occurrence is illustrated in brief in the final two images and table. In the example below a reclassed aspect map (classed into 45 degree increments) is used as the base layer. Sites where diseased individuals occurred can be used to extract information from the base layer. The extracted data can be exported into text for analysis with other software (see final table). Some landscape attributes, such as slope, aspect, elevation, vegetation, distance to water, etc. may be important to disease transmission. These underlying spatial phenomena may guide the movement of animals or disease across the landscape, and may be revealed through the GIS analysis. Interactions between spatial phenomena, such as aspect and another species may be examined using internal multi-variate analytical procedures (linear regression, logistic regression). As mentioned below, statistical assumptions and sampling protocol are key in the interpretation of the results of the spatial data.

Figure 1: Probability of Infection Relative to Distance from Towns

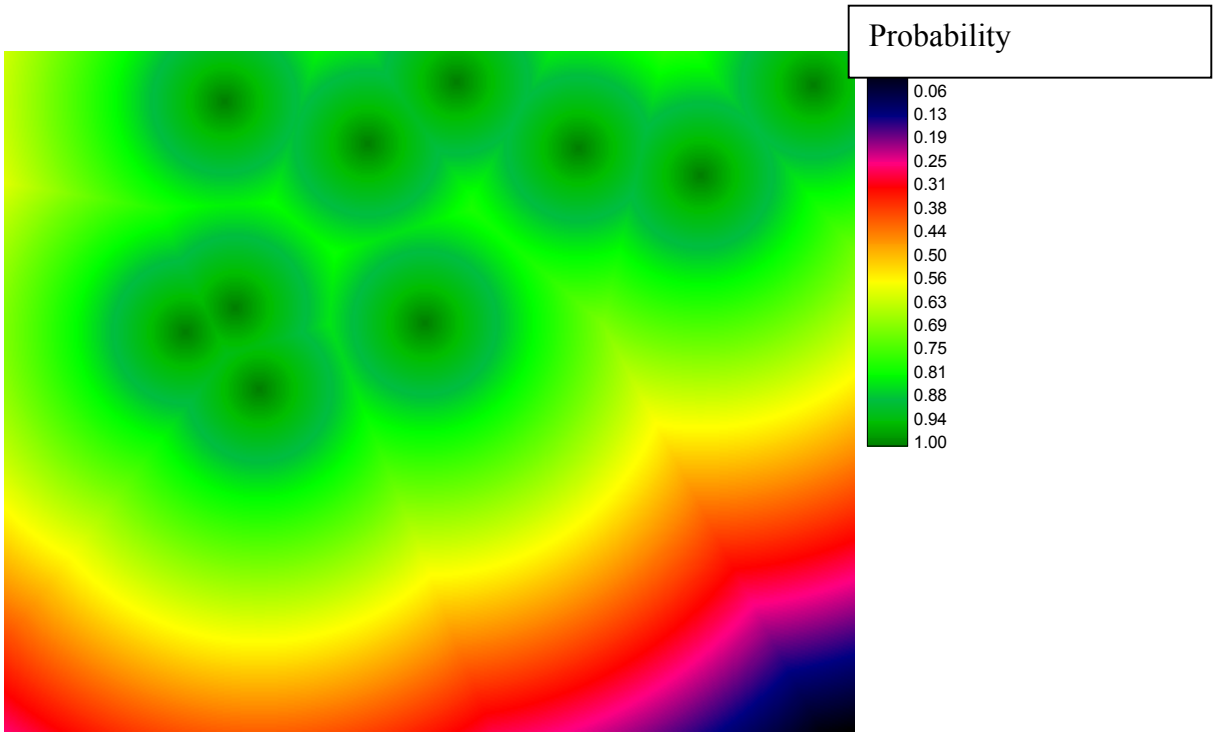


Figure 2: Probability of Infection Relative to Distance from Known Infections

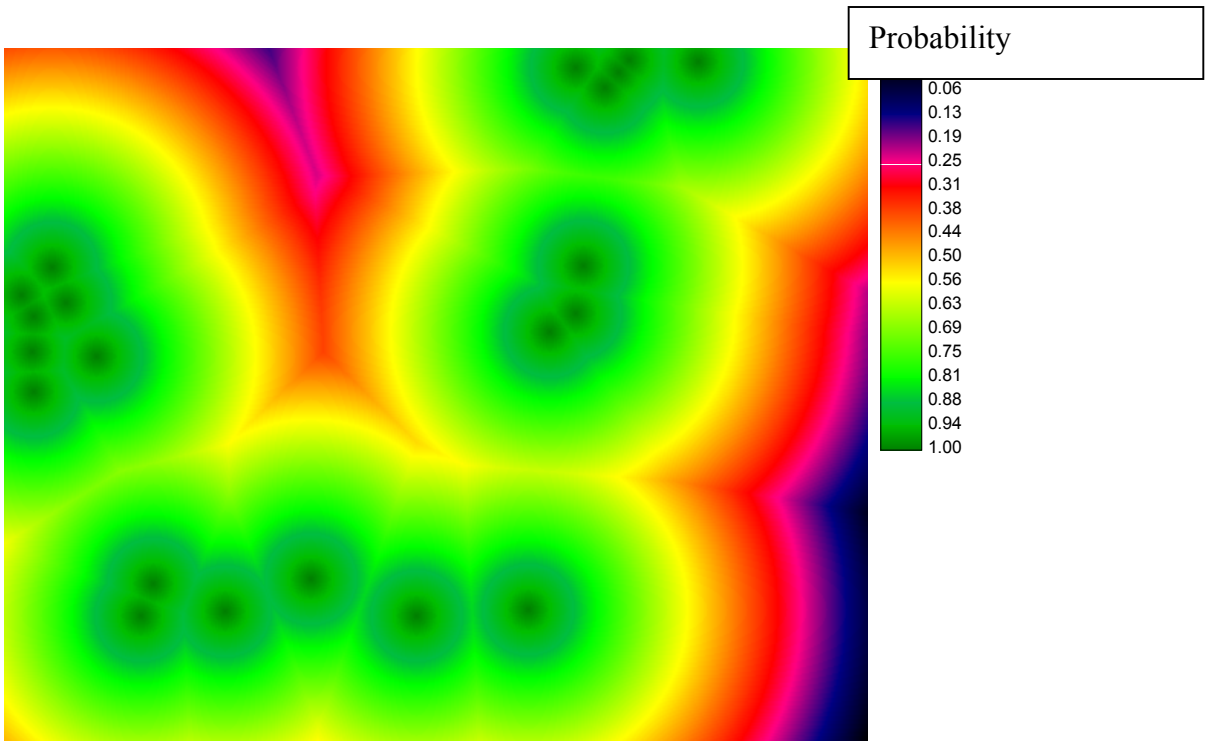


Figure 3: Probability of Infection (Distance from Trail Networks - Vectors of Infection)

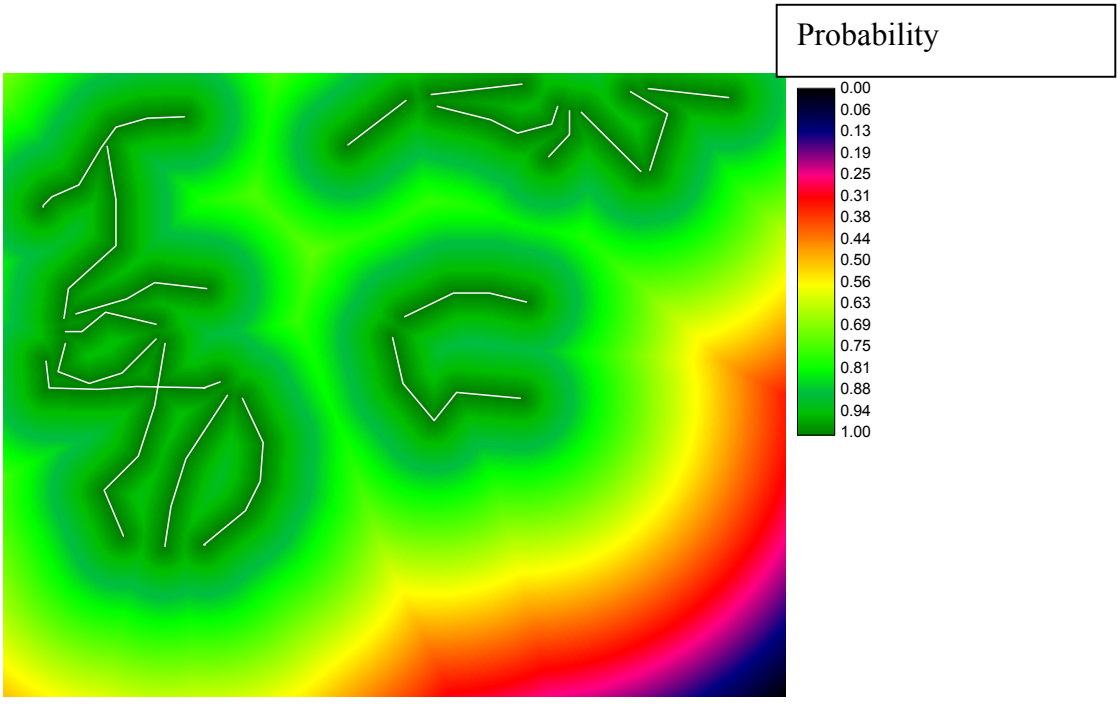


Figure 4: Composite Probability of Infection (Combining All Probability Surfaces)Potential Release Sites are shown as hatched areas, points indicate known infected individuals

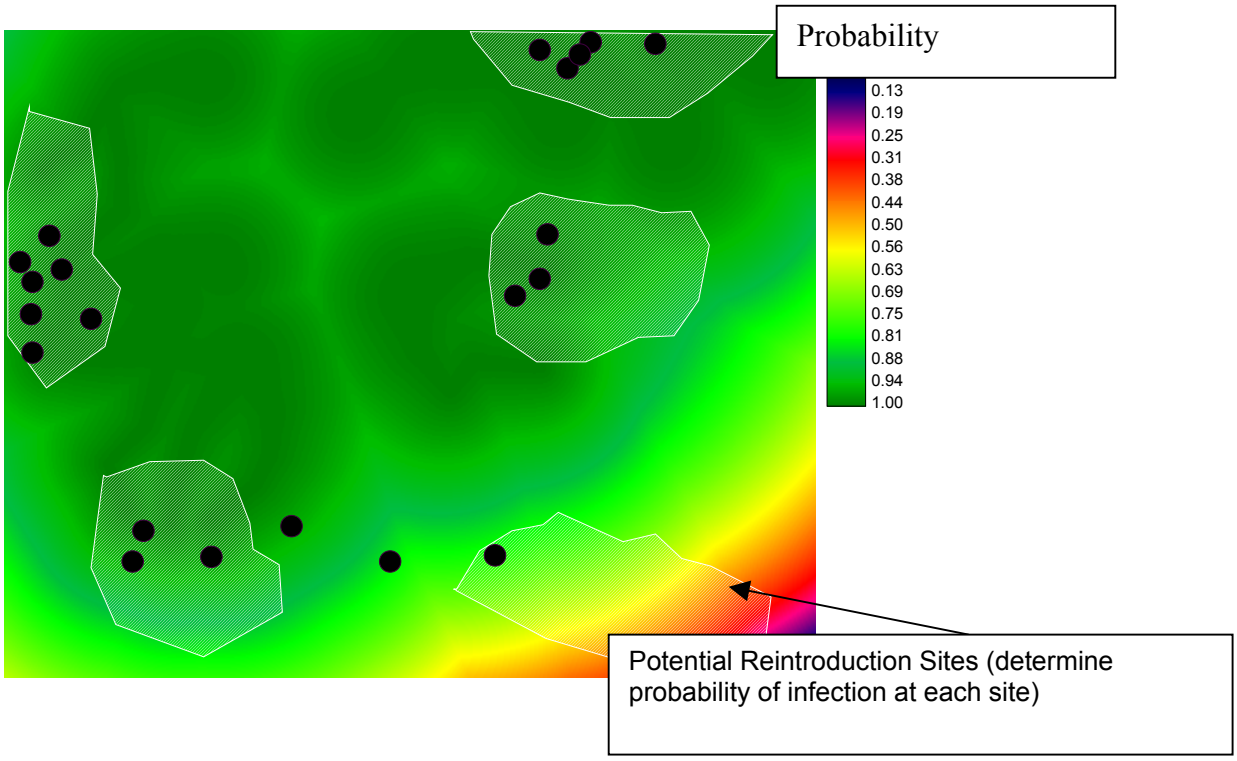
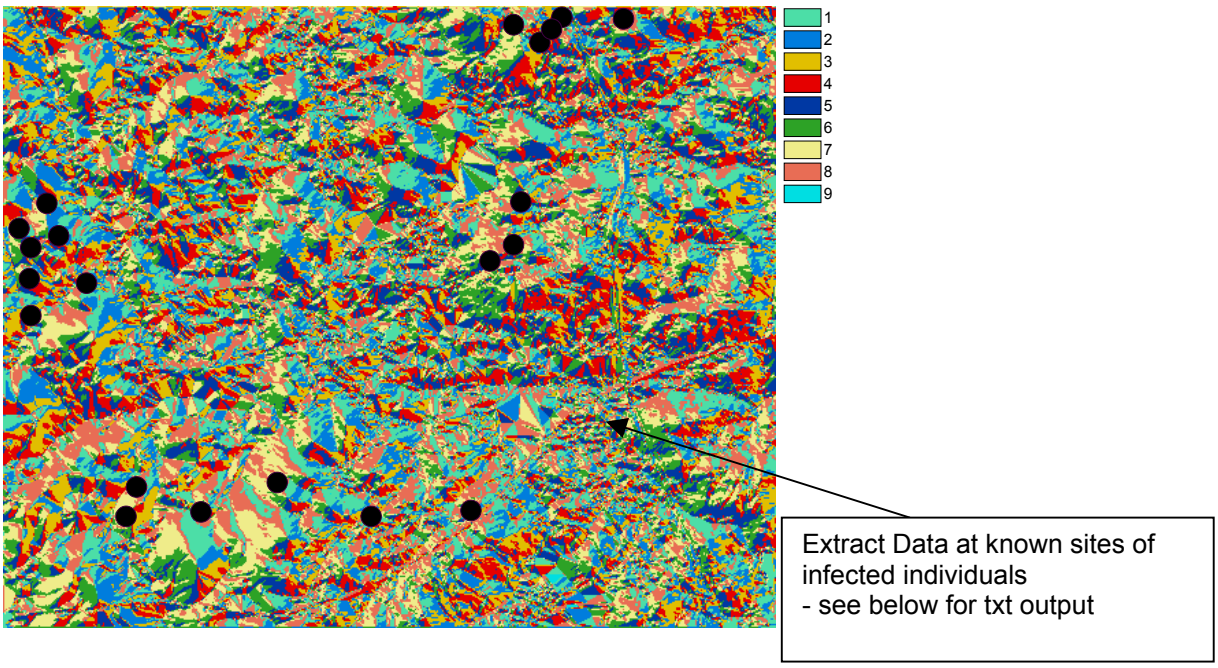


Figure 5: Aspect Surface with Known Diseased Individuals (Black Circles)



Totals extracted from diseased_individuals based on aspect_rcl
(Can be imported into other statistical software

Category	Total
North	3.000000
Northeast	2.000000
East	3.000000
Southeast	1.000000
South	5.000000
Southwest	1.000000
West	3.000000
Northwest	3.000000
Background	0.000000

Statistical analysis may also be conducted within the GIS, however, it is imperative that your spatial sampling of individuals (e.g. diseased bodies) is not biased. For example, if the only individuals found dead are those within 5 km of towns, this may be a function of higher risk of infection near towns, or it may be that areas outside 5km were not surveyed for dead individuals. Be aware of the statistical assumptions of the tests you apply. In many cases, assumptions such as independence are violated by the spatial autocorrelation in sampling.

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IUCN GUIDELINES AND POLICY STATEMENTS

These can be found in English, French, and Spanish on:

http://www.iucn.org/themes/ssc/pubs/policy/index.htm