# V. PATHOLOGY AND EPIDEMIOLOGY

## DISEASE AND THE DIAGNOSTIC PROCESS

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It often is difficult to define health and disease, or to determine where one ends and the other begins. Definitions for these in humans are usually inappropriate for wild species, and death is a rather extreme endpoint to define disease in any species. One way of defining disease is on the basis of impairment of function. This allows consideration of effects on growth, behavior, reproduction, defense and survival as disease. Mild dysfunction, for which the organism can compensate, may fall within relative health, while more severe dysfunction represents disease. Dysfunction can be related to ecological fitness, and the concept can be extended to population effects.

Disease, regardless of cause, begins with injury to individual cells. However, it is the reaction by the organism to cellular injury that results in the dysfunction that we recognize as disease. **Disease is not synonymous with infection or exposure to an agent**; an organism may be exposed or infected but if there is no reaction there will be no disease.

Each organism has only a limited numbers of ways in which it can react to injury. Certain agents elicit a distinct pattern of reaction, but more than one agent may produce the same reaction. Diagnosis is the process of defining those features that distinguish a particular process from all others, i.e. distinguishing disease caused by agent A from that caused by agent B. **Recognition of the pattern of reaction to injury is the basic feature for making a diagnosis in the case of disease**.

The first step in the diagnostic process is to form a working description or **case definition** of the condition. This definition represents the state of knowledge at the time; it is usually crude at the outset, and it becomes progressively refined as information is collected. It is not necessary to know the cause to make a case definition. A critical part of the case definition is characterization of the reaction of the organism to injury. This is the purview of the diagnostic pathologist. While there may be very few "coral pathologists", there is no reason why description and analysis of the reaction to injury should be fundamentally different in coral than in other organisms. **The case definition is the touchstone** ("*a criterion for the quality of a thing*") against which all subsequent portions of an investigation must be tested. For instance, if a condition in another location has a different reaction pattern, or a putative cause results in a different pattern of reaction, one should suspect that the conditions are not the same entity.

A small proportion of diseases are caused by a single agent that is both necessary and sufficient in itself to cause a clearly defined disease. A set of rules (Koch's Postulates) often can be used to establish a cause–effect relationship in this type of disease. However, the great majority of diseases, in all species, are of more complex causation. Agents may cause disease under certain conditions but not under others, multiple agents may be required to produce disease, or several agents may independently cause similar disease. In many diseases there is a complex web of causation that may involve many inter-related factors. Disease must be considered in the context in which it occurs. Koch's Postulates are generally inadequate for establishing cause-effect relationships, and other criteria that include epidemiological information are more appropriate.

Investigation of disease involves answering five basic questions: Who? Where? When? What? and Why? It is impossible to predict in advance which disciplines or diagnostic techniques will be required to solve a problem and no one discipline is omnipotent. However, field and laboratory studies must be related back to the case definition (i.e., Are we looking at the same disease?) and the results should be used to refine the definition of the touchstone.

# EVOLUTIONARY ECOLOGY AND DISEASE EMERGENCE: THE BIG PICTURE

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**ABSTRACT:** Understanding factors responsible for reemergence of diseases believed to have been controlled and outbreaks of previously unknown infectious diseases is one of the most difficult scientific problems facing society today. Significant knowledge gaps exist for even the most studied emerging infectious diseases. Coupled with failures in the response to the resurgence of infectious diseases, this lack of information is embedded in a simplistic view of pathogens and disconnected from a social and ecological context, and assumes a linear response of pathogens to environmental change. In fact, the natural reservoirs and transmission rates of most emerging infectious diseases primarily are affected by environmental factors, such as seasonality or meteorological events, typically producing nonlinear responses that are inherently unpredictable. A more realistic view of emerging infectious diseases requires a holistic perspective that incorporates social as well as physical, chemical, and biological dimensions of our planet's systems. The notion of biocomplexity captures this depth and richness, and most importantly, the interactions of human and natural systems. This article provides a brief review and a synthesis of interdisciplinary approaches and insights employing the biocomplexity paradigm and offers a social–ecological approach for addressing and garnering an improved understanding of emerging infectious diseases. Drawing on findings from studies of cholera and other examples of emerging waterborne, zoonotic, and vectorborne diseases, a ''blueprint'' for the proposed interdisciplinary research framework is offered which integrates biological processes from the molecular level to that of communities and regional systems, incorporating public health infrastructure and climate aspects.

# EMERGING INFECTIOUS DISEASES

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## 1.0 Background

Newly emerging and re-emerging infectious diseases have been of increasing concern over the past 20 years (1-3). Global transportation of people, animals, and food supplies; increased interactions between wildlife, domestic animals and people; high-concentration populations; and the increase in the number people with compromised immune systems (AIDS/HIV and growing elderly populations) have all been identified as risk factors for emerging infectious diseases. This paper summarizes what has been learned from emerging infectious diseases in human and non-human animal populations; and provides preliminary recommendations for responding to emerging infectious diseases in Pacific coral reefs.

## 2.0 Unique profiles of emerging diseases

Emerging infectious diseases, by their nature, affect populations differently than non-emerging diseases. These differences may be considered when developing a strategy to prevent or respond to a population health event involving an emerging disease. The emergence of infectious diseases is most often due to 1) new pathogens, 2) changed indigenous pathogens, or 3) a compromised animal population. When a newly emerging infectious disease is introduced to a population, a large percentage of animals (if not all) may be immunologically naïve to the pathogen. As such, this pathogen is likely to cause rapid morbidity and/or mortality throughout the entire population. The rapid spread of the coronavirus causing Severe Acute Respiratory Syndrome (SARS) in China and other countries in 2003 demonstrates what can happen when a new disease affects an immunologically naïve human population (4). Newly emerging diseases may also affect multiple animal species concurrently; for example, during the Ebola virus outbreak in the Congo Republic in 2003, mortalities were reported in both gorilla and human populations (5); as another example, West Nile virus continues to cause concurrent morbidity and mortality in bird and human populations (6). Compared to newly emerging infectious diseases, re-emerging infectious diseases often occur due 1) a change in underlying factors within a population, or 2) acquired resistance of a pathogen to treatment. Both tuberculosis and toxoplasmosis became emerging infectious diseases due to the increased number of people with compromised immune systems from the AIDS/HIV pandemic. Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as a pathogen of concern due to its acquired resistance to multiple antibiotics. As such, re-emergence of a previously 'quiet' pathogen may indicate a more susceptible animal population or a new ability for the pathogen to resist natural or medical treatments.

For the reasons outlined above, emerging infectious pathogens are more likely to cause more severe disease in larger percentages of animal populations compared to established infectious diseases. Additionally, re-emerging diseases may indicate an immunocompromised population or an old pathogen with 'new tricks' to overcome natural or medical treatments.

**Challenges of emerging diseases**

There are four primary challenges to addressing the emerging disease issue. The first is the need to assess whether a disease is truly emerging or if case numbers are increasing simply due to improved detection and reporting capabilities. The second challenge is detection and characterization of a novel pathogen; if a pathogen is truly novel, identifying the appropriate diagnostic tools for detection can be difficult. Third, initial treatments for emerging diseases may be limited to supportive care and quarantine until the pathogen can be found; if the pathogen is a virus, treatment options will be limited, and a vaccine will not be readily available to prevent a pandemic. Finally, even if a disease is determined to be emerging, the etiological agent is found, and a treatment is identified, there remains a need to assess whether or not the emerging disease is a truly primary disease or if it is secondary to an underlying factor in the animal population. Responses to these challenges are outlined below.

**3.0 Addressing the challenges of emerging diseases**

Public and animal health agencies throughout the world have implemented targeted mitigation strategies to address the challenges of emerging diseases. Below are five activities that are commonly implemented to detect, track, prevent, and respond to emerging diseases. Any combination of these actions, if not already implemented, may be considered to help protect global and regional coral reef populations.

3.1 Determine the baseline for population health. In order to determine if a disease is truly emerging, there is a need to determine the baseline for a population's health. This baseline provides a statistical means of assessing significant differences in populations before, during, and after a potentially emerging disease. Determination of a population health baseline requires long-term collection of standardized health metrics in a population.

3.2 Establish a standardized disease surveillance system that includes both pathogen-specific and syndromic surveillance. The surveillance system should routinely collect and report standardized data related to known diseases of concern. Additionally, it should enable detection and reporting of an emerging disease event in which the cause (etiology) is unknown; many countries use syndromic surveillance (e.g., incidence of respiratory illness or skin lesions) to detect and track emerging diseases. International, centralized surveillance systems are better at detecting emerging diseases compared to multiple, fragmented surveillance systems.

3.3 Implement a robust disease diagnostics program. The more quickly the definitive diagnosis of an emerging disease can be acquired, the better chance

one has to target appropriate mitigation strategies to prevent a catastrophic event. Use of molecular diagnostics (e.g., polymerase chain reaction) has greatly enhanced the ability to rapidly identify and characterize infectious pathogens from clinical samples.

3.4 Conduct formal epidemiological risk assessments. Using the standardized health and disease data collected through a central surveillance system, formal epidemiological risk assessments can be conducted to 1) determine risk factors for an emerging disease, 2) identify appropriate mitigation strategies for specific disease events, and 3) assess the effectiveness of mitigation strategies on population health. These assessments can help determine if a disease is emerging due to a novel pathogen or a compromised animal population.

3.5 Develop a general emergency response plan. In the event that an emerging disease leads to an epizootic of high mortality, an emergency response plan may help to minimize the global impact of the event. Response plans may be more useful if emerging disease events are categorized (e.g., mild, moderate, severe) with corresponding response plans.

## 4.0 Potential action items for discussion

- Determine standardized health metrics that can be routinely collected and reported for coral populations
- Consider where a centralized coral health & disease surveillance system may reside
- Implement a robust molecular diagnostic program based upon polymerase chain reaction (PCR)
- Determine study questions for future epidemiologic risk assessments
- Create emerging disease event categories (e.g., Code Red, Yellow, Green) with corresponding response plans

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# WILDLIFE DISEASE INVESTIGATIONS 101

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## ABSTRACT

Worldwide we are seeing an increased interest in the ecology of disease in wild plants and animals and a concern over whether anthropogenic environmental changes are significantly influencing disease to the detriment of important species. This seems to be occurring across ecosystem boundaries (ie. in cloud forests, woodlands, savannas, deserts, lakes, rivers, and oceans) and with a wide variety of prominent examples (ie. distemper and TB in African lions, chytrid fungus in many species of amphibians, CWD in deer and elk, mycoplasmosis in tortoise, several viruses and whirling disease in salmonids, toxoplasmosis in sea otters, morbiliviruses in seals and dolphins, rickettsial wasting disease in abalone). Although none of these are the subject of this workshop on Coral Disease and Health; the problems, field methods, concepts and lessons learned from investigations of other wildlife diseases were deemed to be useful as an introduction. This paper will provide descriptions of actual wildlife disease investigations in several wildlife species that illustrate the logistical and physical challenges of trying to determine what causes disease process, how basic field information can be refined and approaches refined and rudimentary methods for management developed. Much of the information is drawn from the author's experience, various sources in the wildlife disease and health literature, and Wobeser, 1994.

**INTRODUCTION:** Investigations of wildlife diseases are not really new, they have been conducted for at least a century in North America and longer in Europe and Africa. However, the frequency of investigations, their complexity and importance attached to this area of research and service has increased greatly, particularly in the last decade. Wildlife disease investigations are conducted by State, Federal and tribal governments as part of their stewardship responsibilities for wildlife, by universities and institutes as part of academic or teaching responsibilities, by NGO's and conservation groups attempting to foster species recovery or health, and by cooperatives which are usually hybrids of the proceeding 3 institutional types. Examples of the government agencies might include USFWS and USGS under Department of Interior, NOAA-NMFS and NOS under Commerce, USDA-WS and USDA-APHIS under Agriculture; the Fish and Wildlife Agencies of the 50 States and the Canadian provinces; and some of the larger native American tribes such the Navaho and Yakima. A number of universities in North America have been involved in wildlife health and disease research and many of the first host/agent case descriptions, recognitions of environmental influence on disease and recognition parasite life cycles come out of academic research. In the last few decades nongovernmental organizations like the Wistar, Scripps and Hubbs Institutes, Wildlife Conservation Society, the Morris Animal Foundation and others have funded and supported wildlife disease research. Several large cooperative efforts, notably the

Southeastern Wildlife Disease Cooperative and the Canadian Wildlife Health Cooperative have successfully combined university, government and other resources, mandates and personnel to provide wildlife health research and services.

**Scenario 1**: In February of 1995 a California wildlife veterinarian got a call from the manager of Grizzly Island wildlife area in the Suisun Marsh just north of San Francisco concerning tule elk (*Cervus elaphus nannodes*) dying. These elk, a subspecies that had adapted to the swamps and fens of the central valley and coastal areas of California, had been hunted to near extinction in the 19th century and only remnant populations survived into the mid 20th century. In the 1970's and 1980's groups of tule elk were captured and translocated to a number of State, Federal and privately owned properties. The elk had done very well at this refuge and had surpassed 120 animals on 500 hectares. Several elk had been found down and others were dead. The downed animals appeared to be seizuring, paddling and those that had recovered appeared drunk and disoriented. In all it was estimated that at least 10-12 of the elk were affected and assistance was requested.

**Analysis:** This is a State managed species, on a State refuge, with the contacted person being a State Game and Fish Veterinarian responsible for health and disease in living and dead free-ranging species. No jurisdiction crossed, no permits or plans needed, no significant permission to request either for work on dead animals, handling or "take" of sick. It is an acute unexpected, previously undescribed event or phenomenon, a significant proportion of the population is affected, and immediate response is probably appropriate, although research, planning and sampling gear preparation are limited.

**Response:** Load up the necropsy kit, a rifle, sampling gear, immobilization equipment, leave the wife a note, (that's something people did before cell phones) and hit the road.

**Findings:** Eleven dead animals were located, all of them very fresh having died within the last day or two. All were yearlings, 6 male, 5 female. Several had been paddling around for several hours working up the ground around them, no external lesions of signs other than minor contusions and abrasions noted. Several live animals including at least one adult male were seen showing signs of incoordination, stiff high gait, opisthotonus (neck arched and head held high). Two live elk were down and could not rise. Treatment with steroids, antibiotics (penicillin) and atropine were tried with no results.

**Postmortem examinations were done on 5 of the dead elk.**

Dead elk were in fair to good body condition, no significant lesions were noted in the eyes, ears, nose, or mouth. Brains were not examined in the field. The lungs, heart, liver, kidneys, spleen, adrenals, reproductive organs, intestinal tract also appeared normal. The rumen contained bright green, somewhat frothy contents and some pale carrot like tubers. The smell of the rumen contents was unusual.

There had been many days of fog and rain and most grasses and forbs were buried under a think thatch of dead grasses. The primary green plant available was poison hemlock (*Conium maculatum*) and areas of heavy grazing and pawing to unearth roots were evident. Animals with CNS signs were actually seen returning to hemlock patches to graze. The roots of the hemlock looked very similar to those seen in the rumen of dead

animals, the leaves were the same bright green and the smell of roots and leaves were similar to that unusual small coming from the rumen. Diagnosis of acute hemlock poisoning was confirmed by isolation of conine toxin from rumen contents.

The following **management actions were recommended**: provide attractive grain and alfalfa hay feed it areas away from hemlock. Spray hemlock patches with carrot oil and disk it under ASAP, set up zone guns to scare elk away from hemlock areas where they were eating it, provide more diverse feed, reduce the numbers of elk on the refuge.

**Scenario 2**: In September of 2002 SCWDS gets a call about white-tailed deer (*Odocoileus virginianus*) that are dying around campgrounds in Great Smokey Mountains National Park. The person calling was camping there and describes depressed animals with foam coming from their mouths and bleeding from the rectums. They had described the deer to a NPS Ranger who didn't seem too excited about it and the person, a citizen of Athens, GA., was aware that SCWDS does a lot of wildlife health work all around the southeastern USA.

**Analysis:** SCWDS works with NPS and a call to offer diagnostic assistance and to discuss the need to deal with park visitor concerns is warranted. The description sounds a lot like EHD or bluetongue, an endemic orbiviral disease of deer, which is fairly common in the summer and fall but could be the start of a die off. Call the chief ranger of Great Smokey Mtns., it's their call. They may want to collect and ship the animals or take samples as they have been part of previous sampling programs.

**Response:** NPS personnel took blood samples from fresh dead deer and sent them to SCWDS. SCWDS personnel visited the park a week later and collected 4 deer, and examined 2 of which had died recently.

**Findings:** EHD 1 virus was isolated from tissues of one deer examined by SCWDS and blood of 1 animal sampled by NPS. Lesions in the 2 dead deer were compatible with hemorrhagic disease, these were confirmed by histopathology. Serology showed high antibody titers to EHD 1 and BT 10 viruses.

**Comment:** Although the initial observations (deer dying near campgrounds, frothing at the mouth and bleeding) were somewhat alarming, the syndrome is relatively common in the southeastern USA, particularly in the late summer and fall. The disease is a relatively natural process little influenced by human activities, but the epidemiology is followed closely by SCWDS in hopes of identifying predictors. Few if any actions are recommended for management despite the fact that dieoffs can involve many animals.

**The above scenarios illustrate some reasons why wildlife disease investigations are done.** Basic reasons for studying any disease (from Wobeser 1994) are to:

1) determine its nature and cause,
2) to determine the effects on individuals, populations and ecosystems (to assess its significance),
3) to identify methods to prevent, control or reduce the disease or its effects.

In addition, **with regard to wild species** the reasons may also include:
1) curiosity about the disease as a biological phenomenon,
2) concern over its impact on wild populations and ecosystem integrity,
3) public concern over highly visible die offs or unsightly conditions,
4) concern that disease in a wild species may be transmissible to humans or domestic species, and
5) concern that diseases in wild species are indicators of undesirable changes in the environment.

**Scenario 3:** In April of 1989 California bighorn sheep (*Ovis canadensis californiana*) in the Warner Mountains of the northeastern corner of California are reported to be dying, only few living animals and several bodies were spotted in a recent aerial survey. Mule deer (*Odocoileus hemionus*) in the area are also reported to be dying in large numbers in the valley bottom adjacent to a north-south highway and valley ranch lands.

**Background:** This bighorn population had started from a nucleus 12 animals released into a vast mountain range 10 years previously and was thought to now number approximately 70 individuals. Only about 600 of this subspecies live in California and they are State listed as "threatened". Bighorn sheep have a history of dying in large numbers due to fibrinopurulent (*Pasteurella*) broncho- pneumonia. Previous dieoffs in other locations have resulted in local extinctions. There are serious conservation and political implications (previous dieoffs have been associated with contact between domestic sheep and bighorn). The area is vast and steep, it is a late winter and only the valley areas thaw during sunny days.

**Analysis:** Investigation needs to proceed immediately as decomposition and scavenging will obliterate destroy all evidence quickly. The entire heard of bighorn could be in jeopardy. The deer and bighorn dieoffs may be linked or entirely separate events.

**Response:** Load up the necropsy kit, a rifle, sampling gear, arrange for a helicopter that can do both survey and capture (net-gunning) for sampling, arrange for the ex-wife to care for your dog (divorce is one hazard of lots of field work). Discuss the situation in detail with the local biologist, make arrangements to meet with ranchers in the area who may be cooperative and who control ground access.

**Findings:** Many deer carcasses in various states of preservation (those that die in the shadows have probably been frozen for weeks). Quick field postmortem examinations are done on 5. All the deer (about 10 observed grossly, 5 necropsies) are extremely emaciated. None show significant parasite loads, evidence of infectious disease processes including but not limited to respiratory disease, gastrointestinal disease, systemic lymphoid or hemorrhagic disease. Stomach contents are minimal but often straw or old alfalfa from hay bales. No significant natural browse or forage is available due to prolonged cold winter. General impression is one of starvation/malnutrition.

While doing post mortem examinations of deer at one of the cooperating ranches, the rancher mentioned that he had lost a number of sheep that he grazed in the adjacent range and had only recently found them as they were driven down by harsh weather. He had killed them and allowed access to the reasonably fresh carcass of one. Histological

examination by a state diagnostic lab pathologist revealed chronic bronchitis and low grade bacterial pneumonia. Cultures grew no bacteria of note.

Relatively few bighorn sheep carcasses are found and these have been dead for weeks and are badly scavenged. There are some sites of subcutaneous fat and bone marrow fat is evident when long bones are broken. Mats of yellow fibrin and black discoloration of the pleural lining suggest and portions of one lung suggest fibrinopurulent bacterial pneumonia. A small herd (3) feral goats are spotted in the area and one live bighorn ram is seen running and behaving in ways that suggest it is reasonably healthy. It was captured and sampled (blood, feces, nasal and tonsular swabs), tagged and collared and released. These samples reveal no lungworm (*Protostrongylus spp*.), no evidence of systemic infection (from CBC and chem.), no significant pathogenic bacteria isolated. The three feral goats were shot and postmortem examination was unremarkable. A *Pasteurella multocida* was isolated from tonsil of two animals.

**Summary:** The case described is not unusual in wildlife mortality events. Time, space, terrain, weather and other factors make it very hard to establish a cause of death or to sort out potential causes from coincidental events. The bighorn sheep dieoff pattern is typical of that seen in many western states when bighorn have contact with domestic sheep or goats that may carry pathogenic *Pasteurella* or *Manheimia* bacteria in their upper respiratory tracts. A few shreds of evidence suggest that either the feral goats or the feral sheep may have been involved, as both were in the same general area as the bighorn and both had some evidence of some potentially virulent bacterial respiratory flora, but certainly no cause and effect conclusions can be drawn. Under some circumstances bighorn may develop bacterial pneumonia without contact with domestic sheep or goats. A very few sightings of bighorn sheep in the range persisted for a few years, but all lambs born died before weaned and the herd slipped into extinction within 3 years of the outbreak. The deer dieoff appeared to have nothing to do with the bighorn dieoff but indicated harsh weather conditions that may have impacted other species as well. Without solid evidence on which to base land use policy decisions, enforcement actions, and wildlife management programs it is impossible to manage disease related conflicts between land and resource users.

**Understanding causation is extremely important:** In its simplest form disease may be seen a single agent infecting one species of hosts, relatively uninfluenced by the environment, with clear and decisive outcomes (recovery or death). Clear biological, pathological and pathogen isolation information clarifying the role of host(s), agent and environment make understanding causation much easier. Unfortunately, those kind of simple, straight forward situations are not common.

(From Wobeser 1994) With the discovery of microbial pathogens at the turn of the 20$^{th}$ century, human and veterinary medicine was concerned with identification of specific agents responsible for acute infectious diseases. A set of rules (Koch's postulates) were developed for establishing cause and effect relationships that were generally widely accepted. These were:

1) the agent must be shown to be present in every case of disease through isolation in pure culture,
2) the agent must not be found in cases of other diseases,
3) the agent must be capable of experimentally reproducing the disease, and
4) the agent must be recovered from the experimental host.

But, Robert Koch is dead and our simple concepts of disease have become considerably more complex and encompassing of a much wider array of processes. Perhaps the broadest view yet is that one can see whole ecosystems as "healthy" or "unhealthy" and perhaps identify the reasons why.

A broader set of criteria for establishing causation, reflecting the multifactorial nature of most disease (adapted from Kelly, Thompson and Evans (1986)) is:

1) the hypothesized cause should be distributed in the population or in nature in the same manner as the disease,
2) the occurrence of the disease should be significantly greater in those exposed to the hypothesized cause than in those not exposed,
3) exposure to the hypothesized cause should be more frequent among those with disease than those without , if risk factors are constant,
4) disease should temporally follow exposure to the cause
5) higher doses or longer exposure to the cause should increase disease occurrence
6) for many diseases a spectrum of host responses along a biological gradient from mild to severe should follow exposure
7) other explanations and associations should be eliminated
8) the association between cause and disease should be evident in various populations studied by different methods
9) elimination or modification of exposure to the cause should decrease occurrence of the disease
10) prevention of exposure or modification of the host response (as by vaccination) should decrease or eliminate the disease
11) disease should occur more frequently in experimentally exposed animals than in controls, and
12) all the relationships and findings should make biological sense.

**Scenario 4:** When diseases occur regularly and are a serious threat to the survival of a species the efforts put into diagnosis and management may be larger and more prolonged than outbreak investigations. Biologists have been studying the diseases and causes of death in southern sea otters for over 35 years and for the last 14 years professional veterinary postmortem examinations have been done on all essentially all fresh dead (from 40-100 animals per year). This effort is seen as vital to sea otter recovery and it has allowed the clear description of a number of previously unrecognized or underappreciated disease processes. With an extensive dataset for comparison, mortality events that exceed average can be recognized and quantified and compared to previous years and events. The goal of these efforts is to identify relationships and associations that might lead to improvements in management that benefit the effected populations.

In the spring of 2003 large numbers of dead southern sea otters were picked up along the California coast. Record or near record carcass pickups occurred for several months and an unusual mortality event was declared by USFWS and NOAA. By the end of 2003 the number of sea otters recovered exceeded any previous year, even when numbers were indexed to population growth. No unusual spatial or temporal clusters of mortality were evident. The causes of death found by pathologists were not "unusual" in the sense that the agents and causes were similar in type and proportion to previous years, just greater, in magnitude with the exception that some animals had evidence of domoic acid (amnesic shellfish poisoning) intoxication. In the end it was felt that DA was an additive mortality factor that was behind the record losses.

In the spring of 2004 another dieoff of even greater magnitude occurred. This time the mortalities were clustered in both space and time, occurring around Morro Bay, CA in mid-April to mid-May. Eventually nearly 50 otters, some of which were initially recovered while still alive were collected. All live animals showed severe central nervous system signs including tremors, coma and seizures, and all but one died within a day of stranding. The majority of dead and dying animals had severe generalized lympadenopathy (swollen lymph nodes), multiorgan congestion, pericardial effusion, cardiac mottling and spleenomegaly. Serology suggested some otters had antibodies to *Toxoplasma gondii* but that many more had very high antibody titers to *Sarcocystis neurona*, both of which are protozoal parasites known to kill sea otters. H&E stained sections of effected otters showed severe meningoencephalitis (infection of the brain) classic rosette formations of schizonts typical of *S. neurona* and immunohistochemistry stains were positive. Although it had been known that *S. neurona* could and would kill sea otters previously, cases had been few in number, sporadic and with no particular spatial or temporal pattern that might implicate a source or cause.

**The above should illustrate the importance of establishing a case description:** (From Wobeser 1994) Identifying and defining a disease or disease process: Defining the cause or nature of a disease, or formulating a working hypothesis should be a very early step in every investigation. This is equivalent to a clinician arriving at a tentative diagnosis after examination of a patient (the "what" questions). This definition, often called a "case description" or "case definition" can be dynamic and is very likely to be modified by subsequent information and/or field work. The patterns of temporal and spatial disease occurrence (the "when" and "where" questions) are very important in understanding disease/health conditions in wild species. The disease or disease process is further defined by answering the "who" is questions by defining the population and parameters affected and the "why" question by defining the consistent cycle of the disease (pathogenesis) in the host and the causation.

**SUMMARY:** Although it has been customary to believe that wild animals are generally very healthy and that health is maintained by natural selection, we are beginning to understand that this is perhaps a very simplistic notion. More importantly there are few places on earth where human activities have not upset whatever natural balance existed and/or where human induced changes are not associated with disease in wild animals, wildlife populations and their ecosystems. Although wild animal populations and ecosystems have the ability to respond and to heal themselves, we must understand and in

many cases correct or mitigate the conditions that caused unbalance and/or disease if healthy populations and the be restored.

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