Journal of Experimental Marine Biology and Ecology 362 (2008) 63–70

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Biomedical and veterinary science can increase our understanding of coral disease

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ARTICLE INFO

Article history:
Received 14 September 2007
Received in revised form 15 March 2008
Accepted 22 May 2008

Keywords:
Causation
Coral
Disease
Epizootiology
Pathogenesis
Pathology

ABSTRACT

A balanced approach to coral disease investigation is critical for understanding the global decline of corals. Such an approach should involve the proper use of biomedical concepts, tools, and terminology to address confusion and promote clarity in the coral disease literature. Investigating disease in corals should follow a logical series of steps including identification of disease, systematic morphologic descriptions of lesions at the gross and cellular levels, measurement of health indices, and experiments to understand disease pathogenesis and the complex interactions between host, pathogen, and the environment. This model for disease investigation is widely accepted in the medical, veterinary and invertebrate pathology disciplines. We present standard biomedical rationale behind the detection, description, and naming of diseases and offer examples of the application of Koch's postulates to elucidate the etiology of some infectious diseases. Basic epidemiologic concepts are introduced to help investigators think systematically about the cause(s) of complex diseases. A major goal of disease investigation in corals and other organisms is to gather data that will enable the establishment of standardized case definitions to distinguish among diseases. Concepts and facts amassed from empirical studies over the centuries by medical and veterinary pathologists have standardized disease investigation and are invaluable to coral researchers because of the robust comparisons they enable; examples of these are given throughout this paper. Arguments over whether coral diseases are caused by primary versus opportunistic pathogens reflect the lack of data available to prove or refute such hypotheses and emphasize the need for coral disease investigations that focus on: characterizing the normal microbiota and physiology of the healthy host; defining ecological interactions within the microbial community associated with the host; and investigating host immunity, host-agent interactions, pathology, pathogenesis, and factors that promote the pathogenicity of the causative agent(s) of disease.

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1. Introduction

A considerable body of literature exists on diseases of corals (Rosenberg and Loya, 2004; Sutherland et al., 2004; Harvell et al., 2007). The effects of many of these diseases on the host and host populations are well-documented; however, the causes of many of these diseases are unknown (Weil et al., 2006). This is unfortunate because not knowing the causes of an animal disease significantly hinders its management and prevents detection of direct mechanistic links between occurrence of disease and environmental perturbation. A variety of factors have contributed to this lack of knowledge. For the past three decades, research has been aimed primarily at documenting signs of coral disease *in situ* and at the impacts and demographic consequences of diseases on coral populations through the collection of field data. In this context, coral disease biologists have often used existing published studies on coral disease and existing disease descriptions in lieu of experimental evidence to link a specific etiology with a gross lesion in the field which has led to considerable confusion in the literature (Richardson, 1998; Lesser et al., 2007). More recently, research to elucidate the etiology and pathobiology of coral diseases is gaining momentum with research groups on every continent targeting this challenging problem. Black band disease (Richardson et al., 2007) and *Vibrio*-associated bleaching (Kushmaro et al., 1996) are two examples where the use of a biomedical approach has improved our understanding of fundamental processes that cause disease in corals and of its dynamic nature (Reshef et al., 2006).

In spite of the need for greater understanding of coral pathogenesis, some investigators advocate that future coral disease investigations should place greater emphasis on elucidating environmental cofactors associated with disease rather than on "primary" pathogens (Lesser et al., 2007). Disease is the outcome of complex interactions between the host, causative agent(s), and the environment. This conceptual model was first investigated by Hippocrates (Martin and Martin-Granel, 2006) in the study of diseases of humans and now forms the basis for the study of disease in animals (Martin et al., 1987)

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0022-0981/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jembe.2008.05.011

and plants (Stevens, 1960). The notion that a single one of the three factors in this model may routinely be more significant for disease causation than the other two is not supported by the literature. In this regard we submit a differing position to that offered in a recent article in this journal arguing in favor of an emphasis on investigation of environmental stressors and their role in causing disease (Lesser et al., 2007). While we acknowledge the importance of understanding environmental cofactors, which in some cases significantly predispose or render a coral more susceptible to disease, we recommend a balanced approach that recognizes the importance of biomedical tools to advance our understanding of the interactions between host, agent, and environment. The need for rigorous biomedical approaches to coral disease investigation that consider all 3 components is becoming increasingly important as global patterns in climate warming and deteriorating water quality affect interactions between host, agent and environment.

The methods used to investigate mortality in domestic (Martin et al., 1987) and wild (Wobeser, 1994, 2005, 2007; Roffe and Work, 2005) animals are widely available in the literature. Such methods rely heavily on the biomedical sciences and are applicable to corals. Importantly, disease investigations using biomedical methods do not exclude the significant contribution of environmental factors to susceptibility and pathogenesis, nor do they imply that a pure or primary infection is always the cause. However, such methods emphasize rigorous anatomic descriptions, microbiological and other diagnostic methods, and controlled experimental designs to arrive at the cause(s) and pathogenesis of disease. In this paper, we provide a framework outlining the fundamental processes and principles used to investigate animal diseases and demonstrate their applicability to coral disease investigation.

2. Basic Terminology

The following definitions and terms have been applied to animal disease research for decades and provide an important framework for identifying and describing coral diseases (also see appended glossary). *Disease* is an interruption, cessation or disorder of body functions, systems, or organs (Stedman, 1976). A *syndrome* is the aggregate of signs or symptoms that together comprise disease (Stedman, 1976). Given this hierarchy, and given that one need not know the etiology of something to call it disease, the proposal by Lesser et al. (2007) to employ the term “syndrome” rather than “disease” for coral diseases of unknown etiology is inappropriate. A *sign* is any abnormality associated with disease discoverable by objective examination of the organism (Stedman, 1976). A *lesion* is any injury to tissue or anatomic change associated with disease (Stedman, 1976) and may be part of a clinical sign. The goal of a good description is to enable the reader to visualize the lesion. The following sentence exemplifies how these terms might be used in a gross description of black band disease: The clinical signs of black band disease of scleractinian corals consist of a variably sized area of tissue loss ranging from 1–2 cm to almost the entire colony comprising a well-demarcated area of bare intact skeleton separated from normally appearing tissue by an undulating dark raised (ca. 1 mm) band ranging in width (1–50 mm); the denuded skeleton most distal to the progressing black band front may have a green-yellow hue (microalgal overgrowth), while the skeleton proximal to the band that has been most recently denuded (less than ca. three days) remains clean and white.

For disease to occur, there must be an interaction between three factors: the *agent*, the *host*, and the *environment* (Martin et al., 1987). The host is the organism affected by the disease (e.g., coral). The agent(s) is/are the factor(s) that directly or indirectly cause(s) disease. *Infectious* agents are capable of causing infection and may be transmissible between hosts (Stedman, 1976; Wobeser, 2005); they typically comprise microorganisms such as bacteria, viruses, parasites, and fungi. *Non-infectious* agents are non-living and are not transmissible. Common examples of non-infectious agents include toxins (natural chemicals), toxicants (man-made chemicals), physical factors (e.g trauma, heat, cold), and metabolic, nutritional, or genetic disorders. The environment is considered to be the third leg of the *disease triad* and provides the stage where host-agent interactions occur (Wobeser, 2005). Important in understanding the disease triad is that host, agent, and environment must interact in a precise way for disease to occur (Martin et al., 1987), and that disease is measured in terms of impairment of function along a continuum between absolute health and death (Wobeser, 2005). For example, in *Vibrio*-associated coral bleaching, the mere presence of the *Vibrio* bacterium associated with the coral host is not sufficient to cause production of toxin P and inhibition of photosynthesis of zooxanthellae; adhesion and ingress of the bacterium into the coral requires elevated temperature (Kushmaro et al., 1998; Toren et al., 1998; Rosenberg and Loya, 2004). Recent evidence suggests that coral hosts have successfully developed resistance to bleaching induced by *Vibrio shiloi* (Reshef et al., 2006) illustrating the fact that disease is a dynamic process, and that changes in host, host microbiota, agent, or environment can result in a pathogen causing disease in one instance but not another.

3. The disease investigation process

Investigation of diseases in corals, as in other animals, follows a series of steps (Fig. 1) involving detection of disease, description of the morphological changes in the host associated with the disease (anatomic pathology), determination of the *etiology* or cause of the disease along with description of the pathogenesis or mechanism of disease development. Closure of the circle usually requires further field investigations to understand how the host and etiologic agent interact in their surroundings to facilitate occurrence of disease in a population.

4. Detection of disease

Two common measures of disease in animal populations are *prevalence* and *incidence*. *Prevalence* is the number of diseased individuals

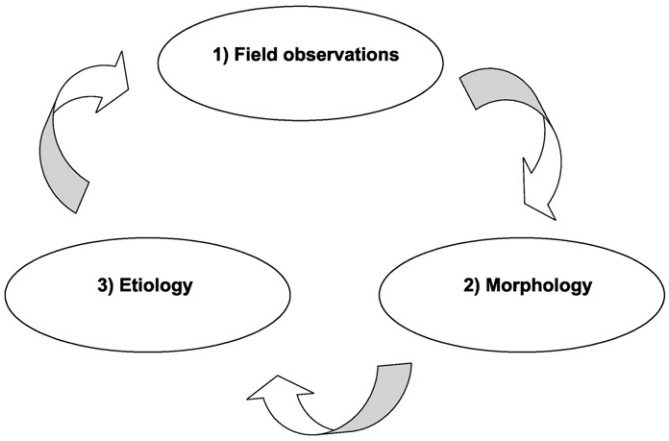

Fig. 1. The circle of disease investigation. 1) A newly emerging disease is first identified by field observations. 2) A systematic morphological description of the observed lesions manifested by the disease is formulated. 3) Laboratory investigations are carried out to identify whether there are potential etiologies, and to understand the interactions between host and agents, including the morphological and physiological (pathogenesis) changes that occur in the host as disease progresses. Hypotheses regarding the agent(s), the host, and their interaction with the environment are formulated and tested through further field observations. Morphological descriptions are refined to the extent possible. Because emerging coral disease outbreaks are typically short-lived when they first appear in a population, it is likely that initial morphological descriptions will need to be refined as additional data are gathered. With disease resurgence, or appearance of an epizootic, new field observations are made to confirm the initial descriptions and validate findings in controlled laboratory settings. Very often, due the transient nature of coral disease outbreaks, the circle is repeated. The information gained from 1–3 above is used to formulate management recommendations and to refine the case definition.

(numerator) divided by the number of diseased plus non-diseased individuals (denominator) *at a point in time* (Stedman, 1976) and is typically expressed as a percentage. *Incidence* is the number of *new* cases of diseased individuals (numerator) *over a defined time period* divided by the number of diseased plus non-diseased individuals surveyed (denominator) (Stedman, 1976). The difference between the two is subtle but important. Prevalence is a static measure encompassing both individuals that became diseased in the past (and continue to survive while exhibiting disease signs) and new cases of disease whereas incidence measures the rate of spread of disease in a population. *Enzootic* diseases are those constantly present in an animal population (Wobeser, 1994). An example of enzootic disease in corals is black band disease that is persistent in some environments (Borger and Steiner, 2005; Page and Willis, 2006; Voss and Richardson, 2006; Barneah et al., 2007). *Epizootic* diseases are those that occur in an unexpected region, time, or place or those diseases that occur at a rate greater than expected based on past experience (Wobeser, 1994). As an example, white band disease (Bythell et al., 2004) can reach epizootic proportions in some regions of the world.

Distinguishing between an epizootic and enzootic disease depends on prior knowledge of its occurrence and requires one to know, at a minimum, the typical prevalence of the disease in that population. A given disease may be enzootic in a population but, under the right demographic or environmental conditions, may reach epizootic proportions. The coral disease white plague type II typically follows this pattern although the environmental cue is not yet known (Bythell et al., 2004). As another example, the protozoal disease of sea urchins caused by *Paramoeba invadens* appears to be enzootic in Nova Scotia and only causes epizootic mortality when seawater temperatures are elevated (Scheibling and Hennigar, 1997). Declaring an epizootic is a somewhat subjective matter of assessing morbidity or mortality relative to rates that are typically expected in the underlying population. Thus, isolation of a virus from two to three cases of morbillivirus-induced disease during an unusual mortality event in endangered Hawaiian monk seals (Osterhaus et al., 1997) could technically be defined as an epizootic, because population size is low and morbilliviral diseases have not been documented in this species in spite of extensive health monitoring over the past 20 years.

5. Description of disease (morphology)

A systematic morphologic description of a disease at the gross and especially at the cellular level is very important. In the case of corals, structured approaches to morphologic descriptions have been addressed by Peters (1984) and Work and Aeby (2006). However, relatively few of the many coral diseases that have been named in the literature have good systematic morphologic descriptions at both the gross and microscopic levels. Determining disease causation may take many years, and causation may be multifactorial and complex. In the interim, precise descriptions of morphology and function are critical to enable comparative studies with similar diseases across species, space, and time.

Morphology, particularly at the cellular (microscopic) and ultrastructural levels (electron microscopy), can also provide clues to organisms that could be associated with disease. For example, in some cases, a potential etiologic agent (bacterium, fungus, parasite, virus) is visibly associated with cellular damage when viewed with either light or electron microscopy. Strong presumptions of association can be inferred in cases where the presumptive etiology is consistently associated with lesions and absent or much reduced in healthy tissue. Note that assumptions about whether an etiology is primary or secondary cannot be made without further study. Morphology associated with a particular disease also changes as disease progresses, and documentation of morphology over time (pathogenesis) is important in order to recognize where a particular lesion may fit within a disease spectrum. Finally, morphology is also useful because it is the name of the disease accompanied by the standardized morphologic description that serves as a *lingua franca* to communicate facts about the disease among researchers.

Disease can also cause alterations in function manifested by changes in biochemical and immunological parameters that may or may not include alterations in morphology. Such changes are readily measured in humans and domestic animals because of the wide availability of laboratory tests and reagents that enable us to detect such changes. Examples include tests that measure both humoral and cell mediated immunity (Glick, 1979), tests that measure changes in organ function such as serum chemistry (Kaneko et al., 1997), and tests that measure biochemical changes such as elevation of certain enzymes resulting from exposure to environmental contaminants like lead (Burch and Siegel, 1971). The development of analogous tests for corals awaits development although progress is being made on the use of biomarkers to assess effects of environmental pollution on corals (Downs et al., 2005). Even with biomarkers, however, morphology will continue to underpin our understanding of coral disease, particular those that are new or emerging.

6. Naming diseases

No set rules exist for naming diseases. Diseases have been named after individuals such as Castleman disease (Casper, 2005), clinical signs manifested by the host such as chronic wasting disease of deer (Williams et al., 2002), morphologic changes induced in the host such as atrophic rhinitis of swine (Pearce and Roe, 1996), or the host and etiologic agent such as avian coccidiosis (Vermeulen, 2004). Names of diseases may evolve over time as more is learned about the disease. For example, "blackhead disease" of turkeys is now also referred to as "avian histomoniasis" after the protozoon (*Histomonas meleagridis*) that causes the disease (McDougald, 2005). All of the aforementioned diseases have well established case definitions (see below) even though in some cases the etiology is unknown (Castleman disease) or caused by multiple organisms (atrophic rhinitis of swine). In summary, unless accompanied by a standardized morphologic description or other defining characteristics, a disease name by itself serves only to introduce confusion into the literature and is essentially useless. In the case of coral diseases, arbitrary assignment of names, use of the same name for different diseases, and use of different names for the same disease have caused much confusion in the literature (Richardson, 1998). Recently, however, a logical formula for naming coral diseases has been proposed (Work and Aeby, 2006). If practical, the formula includes incorporating the affected genus in addition to the generic type of lesion (Work and Aeby, 2006). An example of such nomenclature is *Porites* ulcerative white spot disease (Raymundo et al., 2003).

7. Determining etiology or causation

7.1. Is the disease infectious?

Determining the cause of a lesion in a coral depends on investigations that can range from the simple to the complex. Often, coral disease researchers assume that lesions in corals are due to infectious agents (Lesser et al., 2007), but such assumptions must be tested both observationally and experimentally. Suspicions that a disease may be due to an infectious agent can be gained from microscopic evidence of microorganisms associated with a lesion or temporal field observations suggesting the spread of a lesion from an affected colony to adjacent ones. Ultimately, such suspicions must be confirmed experimentally. A systematic approach that can determine whether an agent is transmissible, whether it is larger or smaller than a virus, and whether it can reproduce the disease in a controlled setting is illustrated in Fig. 2.

If field observations suggest that the disease is transmissible, but disease transmission cannot be replicated in a controlled setting, this

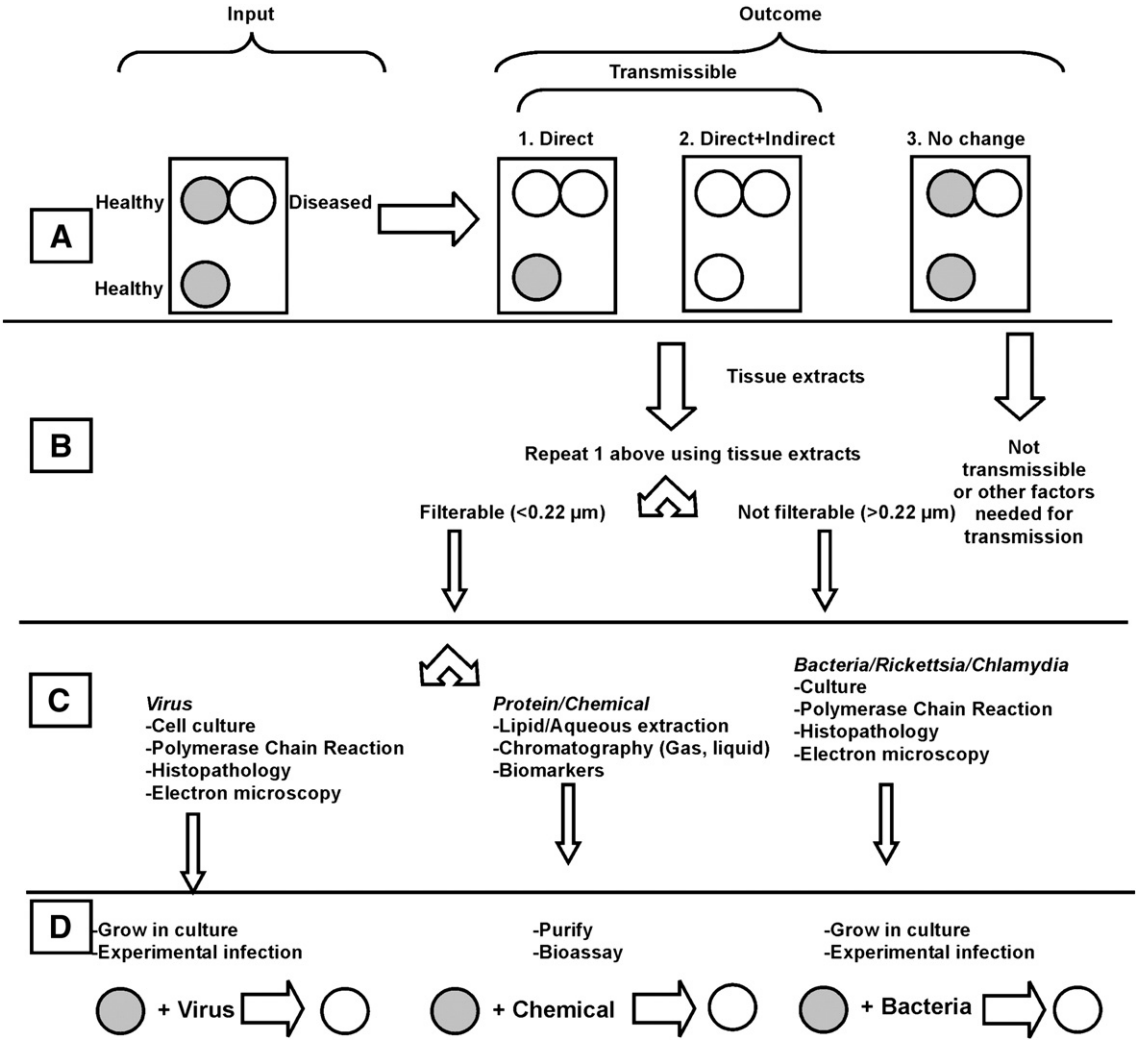

Fig. 2. A) A simple generic experimental setup to determine if a disease is transmissible either through direct or indirect contact. Diseased coral (white circle) is placed in contact with or apart from healthy coral (dark circle) in aquaria. Using appropriate replication and controls, such an experiment can have three explainable outcomes: 1) infectious agent is directly transmissible; 2) infectious agent is directly and indirectly transmissible; or 3) disease is not transmitted. Outcome 3 does not necessarily rule out infectious agents and could be explained by experimental conditions not conducive to disease transmission, or the need for an intermediate host or biological vectors to effect disease transmission. B) If transmission can be replicated experimentally, experiment A is repeated using tissue extracts that are filtered (0.22 µm pore size filter or smaller) or unfiltered (greater than 0.22 µm). Transmissible diseases produced by non filterable agents are generally caused by organisms larger than viruses (bacteria, fungi, rickettsia, etc.) whereas those produced by filterable agents are caused by viruses or subcellular agents (chemicals, proteins, toxins). C) Suspected infectious agents can be detected through various means or grown in culture. Chemicals are purified using biochemical techniques appropriate to the compound. D) In the case of an infectious agent, the putative pathogen is grown in pure culture and further experimental inoculations of the agent using healthy coral (dark circle) are done to fulfill Koch's postulates and attempt to reproduce disease (white circle). In the case of chemicals, bioassays are used to reproduce morphological or physiological changes in the host.

does not necessarily rule out infectious agents. Various factors such as host susceptibility, environmental conditions, and attenuation of the agent may play a role in an inability to replicate an infectious disease in controlled settings. Many infectious agents require intermediate hosts to complete their life cycles. For example, the myxosporean parasite *Myxobolus cerebralis* of salmonids requires an annelid intermediate host to produce the stages that are then infective to susceptible fish (El-Matbouli et al., 1995). In other cases, organisms (vectors) are necessary to transmit critical life history stages of the pathogen from one host to another. Vectors can be *mechanical* in the sense that they serve simply as a vehicle conveying the agent from one host to the next or *biological* where a critical life history stage of the pathogen occurs within the vector. Butterflyfish (Aeby and Santavy, 2006) are an example of a mechanical vector of the polymicrobial community that causes black band disease; by their feeding activity (trauma) fish transport infectious organisms and break the epidermal barrier, part of the host's innate defense, providing a portal of entry for microbial organisms to infect susceptible corals and cause disease. Leeches are an example of a biological vector that transmits protozoal parasites (haemogregarines) to fish (Siddall and Burreson, 1994); in this case, the leech is necessary for the parasite to complete its sexual cycle prior to transmission of the infective stage to the fish host.

7.2. Causation

Causes of disease can be categorized as *necessary and sufficient, necessary but not sufficient, not necessary but sufficient, and not necessary and not sufficient* (Susser, 1991). An example of a cause that is both necessary and sufficient for disease to occur is bovine tuberculosis; the disease cannot occur without the causative bacterium (*Mycobacterium* sp.), and infection of the host with *Mycobacterium* is sufficient to cause disease. For those causes that are not *necessary* or not *sufficient* to cause disease, another set of criteria must be invoked to assign causation (Fig. 3). Fish bites are an example of a cause that is *sufficient but not necessary* to cause acute tissue loss accompanied by skeletal erosion in corals. While fish bites can cause such lesions, abrasions from inanimate objects (anchors, divers) can also cause similar lesions. Although these factors do not represent

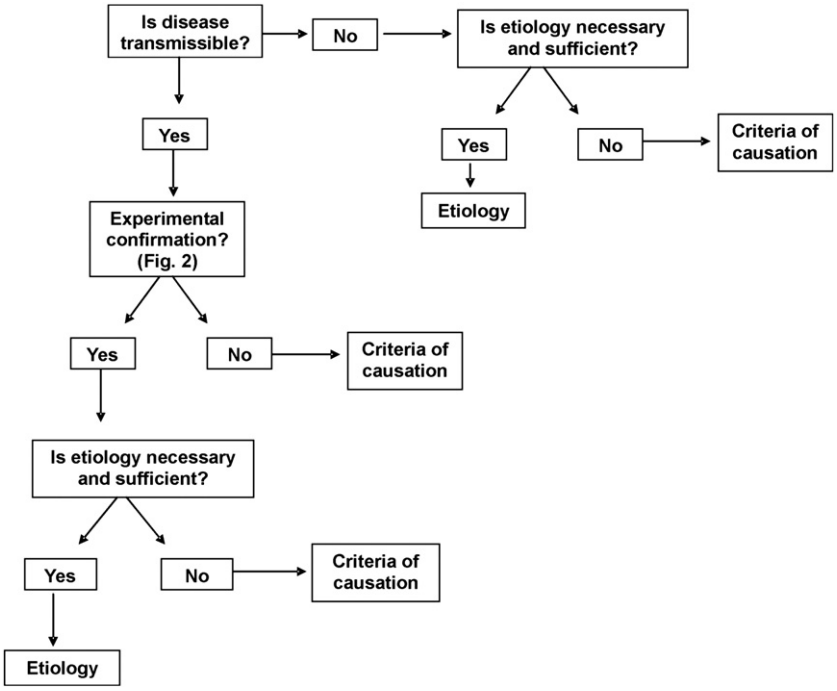

Fig. 3. Conceptual road map to assigning causation in diseases of corals. A hypothesis that a disease is transmissible or not is made based on clues obtained from field observations and morphology. In cases where a cause is identified that is necessary and sufficient for disease to occur, the etiology is identified. In all other cases, criteria of causation must be invoked. Note that three of the five end points invoke criteria of causation illustrating the multifactorial and complex nature of most diseases.

disease agents, they nevertheless cause lesions that compromise the health of the coral. An example of a cause of coral disease that is *necessary but not sufficient* is *Vibrio*-associated bleaching. In this case, the presence of *Vibrio* is necessary, but is not by itself sufficient to cause bleaching because other factors such as temperature and production of toxin P play an important role. The final category (*not necessary and not sufficient*) can be exemplified by any example where a factor has nothing to do with a disease (e.g., number of telephone poles in an urban area and prevalence of heart attacks).

7.3. Epidemiology

Many diseases have complex etiologies. These include polymicrobial diseases with multiple toxins such as black band disease (Richardson et al., 2007), multifactorial diseases such as coral bleaching (Kushmaro et al., 1996; Brown, 1997), or chronic diseases such as green turtle fibropapillomatosis (Herbst, 1994). To better understand diseases having complex etiologies, Hill (1965) invoked criteria of causality which were originally targeted toward clarifying the links between lung cancer and smoking. These criteria were subsequently modified by Susser (1986, 1991) and are as follows:

1) *Strength*: There is a high likelihood or probability that the putative cause is associated with disease.
2) *Specificity*: It can be shown that a particular putative cause leads temporally to a particular disease (specificity in the cause) or a particular disease is associated with a single cause (specificity in effect).
3) *Consistency*: The putative cause can be repeatedly associated with the disease under a variety (replicability) of well defined (survivability) circumstances.
4) *Predictive performance*: A hypothesis derived from an observed association is tested experimentally and yields new information. The observed association must lead to this new information.
5) *Coherence*: The hypothesized causal association is compatible with existing theory (theoretical coherence), preexisting knowledge (factual coherence), biologic knowledge (biologic coherence) and statistical models (statistical coherence).

Both Hill (1965) and Susser (1991) caution that investigators need not slavishly adhere to all criteria, but they do provide a logical framework to think about causal inference in complex multifactorial diseases. A more recent unifying concept is the probability theory of causation (Pearl, 1988; Parascandola and Weed, 2001). Simply put, in probabilistic causation, the component causes of a disease each have individual conditional probabilities that together contribute to the overall probability of effect. The advantage of such an approach is that it forces the investigator to dissect the individual events leading to disease, and it can identify potential data gaps that may require additional investigations.

8. The case definition

The data set (field and laboratory) regarding a disease that is accumulated over time comprises the *case definition* for that disease (Cummings et al., 2001). Importantly, it is not necessary to know the etiology of the disease to develop a case definition (Stedman, 1976). Developing a case definition of a disease is akin to building a house. The foundation is the morphologic description, because it serves as a point of reference for all other investigations regarding the etiology, ecology, and pathogenesis of the disease. As new data are acquired, definitions and morphologic descriptions are refined, more clearly distinguishing the disease from others. Examples of case definitions for various coral diseases are available elsewhere (Work and Aeby, 2006).

9. Are opportunistic pathogens less important than primary ones?

Opportunistic infections are those diseases caused by organisms that do not ordinarily cause disease but that can, under specific circumstances, become pathogenic (Stedman, 1976). Typically, opportunistic infections in animals arise either because the host receives a massive dose of the agent, or the host's immune system (the organism's defense mechanism) is in some way compromised thereby allowing colonization of tissue by the opportunistic pathogen. A classic example in marine organisms is avian aspergillosis that often occurs as a secondary complication in seabirds affected by oil spills. The spores of the fungus,

Aspergillus flavus, are ubiquitous and typically do not cause disease in healthy sea birds. In the case of oil spills, however, birds lose the ability to thermoregulate (oiled feathers) leading to weakening of the immune system and increased susceptibility to opportunists such as *A. flavus*. Often, an environmental component is involved (for example, birds in close quarters being cleaned and rehabilitated with poor ventilation) which exacerbates the problem (Mazet et al., 2002).

Opportunistic pathogens can also cause disease secondarily to the action of a primary agent. For example, organisms that cause "shipping fever" or pneumonic mannheimiosis in cattle include a virus (bovine parainfluenza type 3 virus) that is often the primary agent. Pulmonary macrophages are infected with virus resulting in decreased phagocytosis and killing of bacteria. A secondary infection with a bacterium that is normally part of the host flora, such as *Mannheimia* (*Pasteurella*) *haemolytica A1,* is then favored. Often, the only organism that is observed in an advanced disease state is the bacterium, because the lesions caused by the virus are obscured by the damage done by the bacterial infection. In the case of shipping fever, which is a well characterized disease, the primary agent(s) (viruses) are suspected and can be detected in animals from the same environment with early or no signs of disease. Therefore, the fact that an etiologic agent is opportunistic, or secondary, does not negate the utility of identifying it and elucidating its role in the pathogenesis of disease (Jubb et al., 1993).

A final example is *Vibrio cholera*e, the bacterium that causes cholera in humans. This organism occurs commensally with plankton and shellfish, particularly with copepods. Human cholera outbreaks are seasonal, correlated with water temperatures, salinity, and copepod abundance (Colwell, 2004). *Vibrio cholerae* is "opportunistic" in the sense that it may be ubiquitous in the environment, its toxins are variably produced, its abundance is associated with water temperatures and salinity, and it causes disease when ingested in large doses in drinking water. The case of cholera provides evidence that a disease-causing organism may be normally present in the environment, that the interaction of host and pathogen is often dependent on environmental factors, but that the identification of the agent of disease and its pathogenesis are central to the understanding of such a complex interaction. It is also clear from the study of disease causing organisms such as *V. cholerae* that the term "opportunistic" is somewhat arbitrary because every disease evolves from a complex interaction between the host, agent, and environment.

A recent paper by Lesser et al. (2007) questions the existence of primary pathogens of corals and proposes that most coral diseases are opportunistic thereby justifying an emphasis on investigating environmental factors associated with disease. Arguing over whether diseases are due to opportunistic or primary pathogens misses the more fundamental point which is that we really know little to nothing about the interactions between agent, host, and environment in disease causation in corals. There is a fine line between the effects of a "primary" and "opportunistic" infection which depends largely on the physiologic status of the host, the characteristics of the agent, and the environment in which these two components interact. As pointed out by others (Ritchie, 2006; Reshef et al., 2006; Rosenberg et al., 2007), corals, like other animals such as mammals (Magalhaes et al., 2007) and fish (Roberts, 2001), are holobionts with a microbial community that changes with environmental conditions; this microbial community is likely to play an essential role in the development and progression of disease. However, convincing data do not exist at present to support or refute the proposition that the majority of coral diseases described in the literature are caused by opportunist pathogens (Lesser et al., 2007). Determining whether a pathogen is opportunistic will require investigators to characterize the normal microbiota of the healthy host; define the ecological interactions within the microbial community associated with the host; and assess host immunity and host-agent interactions, host physiology, pathology, and factors that promote the pathogenicity of causative agents. Research using each of these approaches is currently underway in a number of laboratories. Because current understanding of the physiological mechanisms behind many coral diseases is limited, and because efforts to understand these are in early stages, the status of coral disease research is at a critical juncture. Given this limited knowledge, investigators of coral disease should strive to apply equal weight to understanding the agent, the host, the environment, and their interactions and not emphasize one of these three factors as advocated by some (Lesser et al., 2007).

10. Future challenges

Compared to disease investigations in vertebrates, the study of coral disease is in its infancy. Investigations related to pathogenesis of disease in corals are few. The confusion that exists in the literature (Richardson, 1998; Sutherland et al., 2004; Work and Aeby, 2006) will need to be clarified using both biomedical and ecological approaches to investigate and describe diseases. Additional tools will be required to define and investigate diseases and their interactions with the physiology of corals. For example, a variety of cell lines is available to isolate viruses in terrestrial organisms and fish (e.g., the American Type Cell Culture Collection) but such cell lines are lacking for corals. Many commercially available reagents (e.g. antibodies) are either not suitable to study coral disease or were developed to detect proteins specific to domestic vertebrates. Hence, many of these will need to be validated or newly developed. Finally, determining causality using Koch's postulates will only be possible when putative coral pathogens can be isolated and cultured or, when they cannot, by developing modified versions of Koch's postulates that maintain the same rigorous standards. Because many marine microorganisms remain unculturable in the laboratory (Rappe and Giovannoni, 2003), circumventing this obstacle to understanding causality will pose considerable challenges.

Although the obstacles are numerous, a melding of field and laboratory approaches coupled with joint efforts between animal health specialists and coral biologists has considerable potential to move the field forward. Already, collaborative national and international partnerships such as those within the Coral Disease and Health Consortium (Woodley et al., 2003) are bringing together coral biologists, ecologists, veterinarians, microbiologists, veterinary pathologists, molecular biologists and epidemiologists to solve problems in coral disease investigations. Such partnerships enhance our understanding of the role that coral disease plays in coral reef ecosystems and are likely to provide the necessary knowledge to enable us to better understand and eventually manage disease in coral reefs.

Acknowledgments

James Cervino, Ariel Kushmaro, Carol Meteyer, Frank Morado, Esther Peters, Eugene Rosenberg, Garriet Smith, Esti Winter, Cheryl Woodley, and anonymous reviewers provided constructive comments during the development of this manuscript. TM Work also benefited from discussions with Cheryl Woodley and Craig Downs. **[SS]**

Appendix A. SIDEBAR-Koch's postulates

Koch's postulates (1891) can be particularly useful when dealing with potential etiologies that are thought to be necessary and sufficient to cause disease. To fulfill Koch's postulates each of the following must be demonstrated:

a) The etiologic agent occurs in every case of the disease and under circumstances which can account for the pathologic changes and clinical course of the disease.
b) The etiologic agent occurs in no other disease as a fortuitous and non-pathogenic parasite.
c) After being fully isolated from the body and repeatedly grown in pure culture, the etiologic agent can induce the disease anew.
d) The etiologic agent can then be re-isolated from the diseased host.

Three important points regarding fulfillment of Koch's postulates should be emphasized. First, morphologic changes associated with disease serve as the touchstone to aid in elucidation of causation. Second, fulfilling Koch's postulates requires that the suspected etiologic agent be grown as an axenic (pure) culture. For certain microorganisms, particularly those that require cell culture systems such as *Chlamydia*, *Rickettsia*, or viruses, this may not be possible. For corals in particular, the development of cell culture systems is in its infancy (Kopecky and Ostrander, 1999; Domart-Coulon et al., 2001). Additionally, many microorganisms in the marine environment are not culturable in the laboratory by conventional means (Rappe and Giovannoni, 2003). New techniques, however, for cultivation of previously unculturable marine bacteria have recently been developed, and analogous techniques may be of use in coral disease research (Zengler et al., 2002). Third, testing Koch's postulates is challenging for corals because of difficulties in duplicating the environment of the coral host in a laboratory setting.

Refinements of laboratory (aquaria) cultivation methods have improved the ability to successfully maintain corals in the laboratory and to duplicate the natural setting. In spite of this, some have questioned the feasibility of using Koch's postulates in elucidating causality of coral diseases that often have overlapping presentations, and advocated the use of simplified Koch's postulates and molecular tools to identify organisms associated with coral disease (Lesser et al., 2007). We recognize that molecular tools can certainly provide strong evidence that an organism is associated with a disease; however, association does not equal causation. For example, fibropapillomatosis in sea turtles is a tumor-causing disease caused by a filterable agent (Herbst et al., 1995), and compelling molecular evidence indicates that a non-culturable herpesvirus is strongly associated with fibropapillomatosis (Quackenbush et al., 2001). However, it is presently unknown whether tumors in turtles arise because of the herpes viral infection or whether tumorous tissue in sea turtles is somehow permissive to colonization by herpesvirus. Until the virus can be isolated and the disease reproduced experimentally, the relationship between herpesvirus and fibropapillomatosis will remain simply an association and not a cause.

If an infectious etiology of disease is suspected, the importance of attempting to culture the agents suspected of causing disease and of fulfilling Koch's postulates cannot be overemphasized. *Vibrio*-associated bleaching is an example of an infectious coral disease in which Koch's postulates have been fulfilled (Ben-Haim et al., 2003). However, there are also coral diseases in which there is no infectious agent. Two examples are thermal bleaching of corals (Brown, 1997) and fuel oil toxicity in corals (Rougee et al., 2006). In such cases, it becomes necessary to confirm the non-infectious etiology of disease by exposing the organisms to the suspected causes under controlled settings (Fig. 2) and to use appropriate tools to document the pathogenesis of disease (Downs et al., 2005). In the case of fuel oil toxicity, corals that were exposed to various concentrations of fuel oil showed alterations of a number of different physiological parameters (Rougee et al., 2006).

References

Aeby, G.S., Santavy, D.L., 2006. Factors affecting susceptibility of the coral *Montastraea faveolata* to black-band disease. Mar. Ecol. Prog. Ser. 318, 103–110.

Barneah, O., Ben-Dov, E., Kramarsky-Winter, E., Kushmaro, A., 2007. Characterization of black band disease in Red Sea stony corals. Environ. Microbiol. 9, 1995–2006.

Ben-Haim, Y., Thompson, F.L., Thompson, C.C., Cnockaert, M.C., Hoste, B., Swings, J., Rosenberg, E., 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. Int. J. Syst. Evol. Microbiol. 53, 309–315.

Borger, J.L., Steiner, S.C.C., 2005. The spatial and temporal dynamics of coral diseases in Dominica, West Indies. Bull. Mar. Sci. 77, 137–154.

Brown, B., 1997. Coral bleaching: causes and consequences. Proc. 8th Int. Coral Reef Symp., vol. 1, pp. 65–74.

Burch, H.B., Siegel, A.L., 1971. Improved method for measurement of delta-aminolevulinic acid dehydratase activity of human erythrocytes. Clin. Chem. 17, 1038–1041.

Bythell, J.O., Pantos, O., Richardson, L.L., 2004. White plague, white band, and other "white" diseases. In: Rosenberg, E., Loya, Y. (Eds.), Coral Health and Disease. Springer, Heidelberg, pp. 351–364.

Casper, C., 2005. The aetiology and management of Castleman disease at 50 years: translating pathophysiology to patient care. Br. J. Haematol. 129, 3–17.

Colwell, R., 2004. Infectious disease and environment: cholera as a paradigm for waterborne disease. Int. Microbiol. 7, 285–289.

Cummings, S.R., Browner, W.S., Grady, D., Hearst, N., Newman, T.B., Hulley, S.B. (Eds.), 2001. Designing Clinical Research: An Epidemiologic Approach. Lippincott Williams & Wilkins. 336 pp.

Domart-Coulon, I., Elbert, D.C., Scully, E.P., Calimlim, P.S., Ostrander, G.K., 2001. Aragonite crystallization in primary cell cultures of multicellular isolates from a hard coral *Pocillopora damicornis*. Proc. Natl. Acad. Sci. 98, 11885–11890.

Downs, C.A., Woodley, C.M., Richmond, R.H., Lanning, L.L., Owen, R., 2005. Shifting the paradigm of coral reef "Health" assessment. Mar. Pollut. Bull. 51, 486–494.

El-Matbouli, M., Hoffmann, R.W., Mandok, C., 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage. J. Fish Biol. 46, 919–935.

Glick, B., 1979. The avian immune system. Avian Dis. 23, 282–289.

Harvell, D., Merkel, S., Jordan-Dahlgren, E., Rosenberg, E., Raymundo, L., Smith, G., Weil, E., Willis, B., 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography 20, 172–195.

Herbst, L.H., 1994. Fibropapillomatosis of marine turtles. Ann. Rev. Fish Dis. 4, 389–425.

Herbst, L.H., Jacobson, E.R., Moretti, R., Brown, T., Sundberg, J.P., Klein, P.A., 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. Dis. Aquat. Org. 22, 1–12.

Hill, A.B., 1965. The environment and disease: association or causation? Proc. Roy. Soc. Med. 58, 295–299.

Jubb, K.V.F., Kennedy, P.C., Palmer, N., 1993. 4 ed. Pathology of Domestic Animals, vol. 2. Academic Press Inc., San Diego, pp. 613–615.

Kaneko, J., Harvey, J.W., Bruss, M.L., 1997. Clinical biochemistry of domestic animals. Academic Press, San Diego. 932 pp.

Koch, R., 1891. Uber bacteriologishe, Forschung Verhandlung des X Internationalen Medichinischen Congresses. Hirschwald, Berlin, p. 35.

Kopecky, E.J., Ostrander, G.K., 1999. Isolation and primary culture of viable multicellular endothelial isolates from hard corals. In Vitro Cell Dev. Biol. Anim. 35, 616–624.

Kushmaro, A., Loya, Y., Fine, M., Rosenberg, E., 1996. Bacterial infection and coral bleaching. Nature 380, 396.

Kushmaro, A., Rosenberg, E., Fine, M., Ben-Haim, Y., Loya, Y., 1998. Effect of temperature on bleaching of the coral *Oculina patagonica* by Vibrio AK-1. Mar. Ecol. Prog. Ser. 171, 131–137.

Lesser, M.P., Bythell, J.C., Gates, R.D., Johnstone, R.W., Hoegh-Guldberg, O., 2007. Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. J. Exp. Mar. Biol. Ecol. 346, 36–44.

Magalhaes, J.G., Tattoli, I., Girardin, S.E., 2007. The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens. Semin. Immunol. 19 (2), 106–115.

Martin, P.V.M., Martin-Granel, E., 2006. 2,500-year evolution of the term epidemic. Emerg. Infect. Dis. 12 (6), 976–980.

Martin, S.W., Meek, A.H., Willerberg, P., 1987. Veterinary epidemiology, principles and methods. Iowa State University Press, Ames. 343 pp.

Mazet, J.A.K., Newman, S.H., Gilardi, K.V.K., Tseng, F.S., Holcomb, J.B., Jessup, D.A., Ziccardi, M.H., 2002. Advances in Oiled Bird Emergency Medicine and Management. J. Avian Med. Surg. 16, 146–149.

McDougald, L.R., 2005. Blackhead disease (histomoniasis) in poultry: a critical review. Avian Dis. 49, 462–476.

Osterhaus, A., Groen, J., Niesters, H., van de Bildt, M., Martina, B., Vedder, L., Vos, J., van Egmond, H., Sidi, B.A., Barham, M.E.O., 1997. Morbillivirus in monk seal mortality. Nature 388, 838.

Page, C., Willis, B., 2006. Distribution, host range and large-scale spatial variability in black band disease prevalence on the Great Barrier Reef, Australia. Dis. Aquat. Org. 69, 41–51.

Parascandola, M., Weed, D.L., 2001. Causation in epidemiology. J. Epidemiol. Comm. Health 55, 905–912.

Pearce, H.G., Roe, C.K., 1996. Infectious porcine atrophic rhinitis: a review. Can. Vet. J. 7, 243–251.

Pearl, J., 1988. Causality: Models, reasoning, and inference. Cambridge University Press, New York. 384 pp.

Peters, E.C., 1984. A survey of cellular reactions to environmental stress and disease in Caribbean scleractinian corals. Helgo. Meeresunters. 37, 113–137.

Quackenbush, S.L., Casey, R.M., Murcek, R.J., Paul, T.A., Work, T.M., Limpus, C.J., Chavez, A., DuToit, L., Perez, J.V., Aguirre, A.A., Spraker, T.R., Horrocks, J.A., Vermeer, L.A., Balazs, G.H., Casey, J.W., 2001. Quantitative analysis of herpesvirus sequences from normal and fibropapillomas of marine turtles with real time PCR. Virology 287, 105–111.

Rappe, M.S., Giovannoni, S.J., 2003. The uncultured microbial majority. Ann. Rev. Microbiol. 57, 369–394.

Raymundo, L.J., Harvell, C.D., Reynolds, T.L., 2003. *Porites* ulcerative white spot disease: description, prevalence, and host range of a new coral disease affecting Indo-Pacific reefs. Dis. Aquat. Org. 56, 95–104.

Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, Il., Rosenberg, E., 2006. The coral probiotic hypothesis. Environ. Microbiol. 8, 2068–2073.

Richardson, L.L., 1998. Coral diseases: what is really known? TREE 13, 438–443.

Richardson, L.L., Sekar, R., Myers, J.L., Gantar, M., Voss, J.D., Kaczmarsky, L., Remily, E.R., Boyer, G.L., Zimba, P.V., 2007. The presence of the cyanobacterial toxin microcystin in black band disease of corals. FEMS Microbiol. Lett. 272, 182–187.

Ritchie, K.B., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar. Ecol. Prog. Ser. 322, 1–14.

Roberts, R.J., 2001. Fish pathology. W.B. Saunders, London. 472 pp.

Roffe, T.J., Work, T.M., 2005. Wildlife health and disease investigations. In: Braun, C.E. (Ed.), Techniques for wildlife investigations and management. The Wildlife Society, Bethesda, pp. 616–631.
Rosenberg, E., Koren, O., Reshef, L., Efrony, R., Zilber-Rosenberg, I., 2007. The role of microorganisms in coral health, disease and evolution. Nat. Rev. 5, 355–362.
Rosenberg, E., Loya, Y., 2004. Coral health and disease. Springer, Heidelberg. 488 pp.
Rougee, L., Downs, C.A., Richmond, R.H., Ostrander, G.K., 2006. Alteration of normal cellular profiles in the scleractinian coral (*Pocillopora damicornis*) following laboratory exposure to fuel oil. Environ. Toxicol. Chem. 25, 3181–3187.
Scheibling, R.E., Hennigar, A.W., 1997. Recurrent outbreak of disease in sea urchins *Strongylocentrotus droebachiensis* in Nova Scotia: evidence for a link with large-scale meteorologic and oceanographic events. Mar. Ecol. Prog. Ser. 152, 155–165.
Siddall, M.E., Burreson, M.E., 1994. The Development of a Hemogregarine of *Lycodes raridens* from Alaska in Its Definitive Leech Host. J. Parasitol. 80, 569–575.
Stedman, T.L., 1976. Stedman's Medical Dictionary. Williams and Wilkin Company, Baltimore. 1678 pp. (http://www.stedmans.com/).
Stevens, R.B., 1960. In: Horsfall, J.G., Dimond, A.E. (Eds.), Plant Pathology, an Advanced Treatise, vol. 3. Academic Press, NY, pp. 357–429.
Susser, M., 1986. The logic of Sir Karl Popper and the practice of epidemiology. Am. J. Epidemiol. 124, 711–718.
Susser, M., 1991. What is cause and how do we know one? A grammar for pragmatic epidemiology. Am. J. Epidemiol. 133, 635–648.
Sutherland, K.P., Porter, J.W., Torres, C., 2004. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar. Ecol. Prog. Ser. 266, 273–302.
Toren, A., Landau, L., Kushmaro, A., Loya, Y., Rosenberg, E., 1998. Effect of temperature on adhesion of *Vibrio* strain AK-1 to *Oculina patagonica* and on coral bleaching. Appl. Env. Micro. 64, 1379–1384.
Vermeulen, A.N., 2004. Avian coccidiosis: a disturbed host-parasite relationship to be restored. Symp. Soc. Exp. Biol. 55, 211–241.
Voss, J.D., Richardson, L.L., 2006. Coral diseases near Lee Stocking Island, Bahamas: patterns and potential drivers. Dis. Aquat. Org. 69, 33–40.
Weil, E., Smith, G., Gil-Agudelo, D.L., 2006. Status and progress in coral reef disease research. Dis. Aquat. Org. 69, 1–7.
Williams, E.S., Miller, M.W., Kreeger, T.J., Kahn, R.H., Thorne, E.T., 2002. Chronic wasting disease of deer and elk: a review with recommendations for management. J. Wildl. Man. 66, 551–563.
Wobeser, G.A., 1994. Investigation and Management of Disease in Wild Animals. Plenum Press, New York. 265pp.
Wobeser, G.A., 2005. Essentials of disease in wild animals. Blackwell Publishing, Oxford. 243 pp.
Wobeser, G.A., 2007. Disease in Wild Animals, Investigation and Management. Springer, Berlin. 393 pp.
Woodley, C.M., Bruckner, A.W., Galloway, S.B., McLaughlin, S.M., Downs, C.A., Fauth, J.E., Shotts, E.B., Lidie, K.L., 2003. Coral Disease and Health: A National Research Plan. National Oceanic and Atmospheric Administration, Silver Spring, MD. 72 pp.
Work, T.M., Aeby, G.S., 2006. Systematically describing gross lesions in corals. Dis. Aquat. Org. 70, 155–160.
Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M., 2002. Cultivating the uncultured. Proc. Natl. Acad. Sci. 99, 15681–15686.

Glossary

Case: Instance of a disease with its attendant circumstances (Stedman, 1976).
Case definition: Data set including but not limited to morphology, clinical signs, physiological, behavioral, epidemiological, and other applicable parameters that characterize a particular disease (Cummings et al., 2001).
Disease: An interruption, cessation or disorder of body functions, systems, or organs regardless of etiology (Stedman, 1976).
Enzootic: Diseases that are constantly present in an animal population (Wobeser, 1994).
Epizootic: Diseases that occur in an unexpected region, time, or place or those diseases that occur at a rate greater than expected based on past experience (Wobeser, 1994).
Etiology: Causation or cause of disease (Stedman, 1976).
Incidence: Percent of new cases of diseased individuals (numerator) over a defined time period (Stedman, 1976).
Infectious: Capable of causing infection (Wobeser, 2005); Capable of being transmitted between hosts (Stedman, 1976).
Lesion: Any injury to tissue or anatomic change associated with disease; may be part of a clinical sign (Stedman, 1976).
Opportunistic infections: Diseases caused by organisms that do not ordinarily cause disease but that can, under specific circumstances, become pathogenic (Stedman, 1976).
Pathogenesis: The mode of development of disease (Stedman, 1976).
Pathology: Study of the essential nature, causes, structural, and functional changes associated with disease (Stedman, 1976).
Prevalence: Percent of diseased individuals at a point in time (Stedman, 1976).
Sign: Any abnormality associated with disease discoverable by objective examination of the organism (Stedman, 1976).
Syndrome: Aggregate of signs or symptoms that together comprise disease (Stedman, 1976).
Vector: Any living organism that transports an infectious agent between hosts (Stedman, 1976).