Disease in Wild Animals: Investigation and Management

2nd Edition

Gary A. Wobeser

Disease in Wild Animals
Investigation and Management

2nd Edition

With 17 Figures

Springer

Prof. Dr. Gary A. Wobeser
Department of Veterinary Pathology
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, Saskatchewan, S7N 5B4
Canada
e-mail: gary.wobeser@usask.ca

The first edition "Investigation and Management of Disease in Wild Animals" (ISBN 0-306-44703-7) was published by Plenum Press, New York, 1994.

Library of Congress Control Number: 2006940351

ISBN-10: 3-540-48974-6 Springer Berlin Heidelberg New York
ISBN-13: 978-3-540-48974-0 Springer Berlin Heidelberg New York

Springer is a part of Springer Science+Business Media

springer.com

Editor: Dr. Dieter Czeschlik, Heideiberg, Germany
Desk editor: Dr. Andrea Schlitzberger, Heidelberg, Germany
Cover design: WMXDesign GmbH, Heidelberg, Germany
Production and typesetting: SPi Publisher Services

Printed on acid-free paper SPIN 11562528 31/3100 5 4 3 2 1 0

Preface

This book arose out of teaching graduate and undergraduate classes in wildlife diseases. It, in some ways, chronicles my involvement in the investigation and diagnosis of diseases in free-ranging wildlife, primarily in western and northern Canada, since the 1960s. It also, perhaps, reflects the development of wildlife disease study as a discipline. Much of the earlier work in this field was purely descriptive, documenting the occurrence of various diseases in wild animals. I have chosen to retain references to some older and obscure information in this second edition because this body of work provides the foundation for a more analytical approach. The literature on health problems in free-ranging animals is expanding rapidly. I am gratified that the theoretical and quantitative aspects of wildlife disease are receiving more attention than in the past, and that role of disease as a factor in population biology is being analyzed. My hope for the first edition of this book was that it would serve as an overview of the study of disease in wild animals and of methods that might be used to manage health problem. It was, and is, not intended to be a how-to book or an encyclopedic reference to the literature on disease; rather it is intended as a seed crystal around which the reader can build. The inquiries I have received about a second edition suggest that it has been useful. The field of wildlife diseases is an interface area between medicine and applied biology. During the past half-century, medical science has become preoccupied with technology and with dissecting disease phenomena at the molecular level in the laboratory. This has resulted in marvelous tools for the study of disease agents. However, study of disease in whole animals and of the population biology of disease became unfashionable, even though such knowledge is essential if the results of high-tech research are to be applied. In contrast, wildlife biology is concerned with populations and, to the wildlife manager, disease is important only when it has an impact on the population. Some basic concepts of epidemiology, such as mortality rate and survival rate of a population, are used more frequently by the average biologist than by the average health practitioner. Medical scientists don't think of disease in terms of fitness, trade-offs or compensation, but these concepts are fundamental to the ecologist. The role of the "wildlife disease specialist" is to use the tools of biomedical science within an ecological framework to understand how and why disease occurs in free-living populations and when and how it might be managed.

I thank my wife Amy Grace for her patience and support; my colleagues in the Department of Veterinary Pathology, Western College of Veterinary Medicine, and the Canadian Cooperative Wildlife Health Centre for allowing me to pursue my interests; the students who have tolerated my enthusiasm and served as a sounding board for notions; and the many wildlife biologists I have worked with over the years who have helped me to keep the importance of disease in perspective.

Saskatoon, December 2006 *Gary A. Wobeser*

Contents

Section I
Introduction

"*Up to the present time it has been customary to believe that wild animals possess a high standard of health, which is rigidly maintained by the action of natural selection, and which serves as the general, though unattainable, ideal of bodily health for a highly diseased human civilization. This belief is partly true and partly false.*"

(Elton 1931)

1 Disease and epizootiology—basic principles

> "*Typically, diseases of wildlife have been investigated by performing pathological examinations on carcasses that are found incidentally, or producing lists of parasites identified in small samples of host species. There have been few attempts to assess the impact of a disease on the host population rather than the individual, or to describe the distribution of the disease agent in a manner sufficient to understand its epidemiology*."
>
> (Gulland 1995)

1.1 Disease and diseases

The concept of disease is surprisingly difficult to define in terms that are sufficiently broad for application to the wide range of conditions that occur in free-ranging wild animals, and that are still sufficiently narrow to separate disease from other factors, such as predation and food supply, that affect wildlife negatively. Disease might be defined as any departure from health, but this leads to a circular discussion of the meaning of health and normality. Disease in wild animals is often considered only in terms of death or obvious physical disability, probably because these are readily identified parameters. However, the effect of disease on wild populations may be much greater than is evident by simply counting the dead or maimed, even if it were possible to do so accurately. The impact of DDT and certain other chlorinated hydrocarbon insecticides on some raptorial and piscivorous birds provides an excellent illustration of this fact. These compounds have low direct toxicity and rarely result in the death of birds or in obvious clinical signs of intoxication, yet they had profound population effects through decreased recruitment as a result of increased egg breakage.

Disease conditions should not be dismissed as inconsequential simply because they occur commonly, nor should one assume that a disease condition or parasite has a major effect on the host simply because it is conspicuous. Infection with parasites of various types is ubiquitous in wild animals and reference is often made to *normal* parasite burdens, the inference being that parasites at this level have little or no impact on the animal. However, the actual effect of these parasites on the host is largely unknown. "*Although most infectious agents do not result in obvious disease, the host must pay a 'price' for harboring parasites that live, grow, and reproduce at the expense of the host*" (Yuill 1987). In domestic livestock, this price can be measured in

terms of decreased efficiency of production and, even in livestock, the true extent of the cost is often not recognized until the parasite or disease has been eliminated. This type of assessment is seldom possible in free-ranging animals but observations from semi-free-ranging animals, of the same species, such as those on game-farms, provide some indication of the cost of parasitism. For example, Szokolay and Rehbinder (1984) reported a 20% increase in the growth rate of fallow deer after gastro-intestinal parasites, of the type common in wild deer, were partially controlled through the use of anthelmintics. Perhaps even more pertinent is the finding that treatment with anthelmintics resulted in an "*almost 100% increase in body weight in the fawns*" and increased antler growth in males among free-ranging roe deer (Duwel 1987). Special techniques may be required to assess the cost of a disease. For example infection with avian malaria (*Plasmodium pediocetii*), while not causing obvious illness in male sage grouse, had a significant effect on breeding that was only detected in detailed behavioral studies. Infected males attended the lek less regularly, copulated less frequently, and bred later in the season, with less "*fit*" females, than did non-infected males (Johnson and Boyce 1991). Female sage grouse selected against mating with males that had hemorrhagic spots on their air sacs of the type produced by lice (Spurrier et al. 1991). We currently do not have sufficient techniques for measuring effects such as subtle alterations in behavior or diminished intelligence that have been documented to occur in parasitized humans (Levav et al. 1995; Flegr et al. 2003).

The effect of disease may only be evident under certain conditions. For instance, infection with blow fly larvae (*Protocalliphora* sp.) had no effect on the weight, size, or age at fledging of young sage thrashers; however, parasitized birds had a higher mortality rate than unparasitized fledglings during a period of wet, cold weather, suggesting an interaction between parasitism and other stressors (Howe 1992). Similarly, Murray et al. (1997) found a synergy between intestinal parasites and nutrition in snowshoe hares when food was limited. It also is important to examine the correct portion of the population in evaluating the effect of disease. Iason and Boag (1988) failed to find any correlation between intensity of infection with an intestinal parasite and body condition or fecundity of adult mountain hares but suggested that it would be very important to determine the effect of the parasite on growth and survival of young hares before concluding that it was harmless. The members of any population are not homogenous and a small proportion of the population may bear the brunt of a disease. This is most clearly established for infections by larger parasites in which "*most hosts have very low parasite burdens and a few hosts have very high burdens*" (Shaw et al. 1998) but the same principle likely applies to many other diseases in which underprivileged individuals in the population are affected disproportionately. It may be very difficult to detect or measure the impact of disease in these situations because severe effects on a small number of animals may have relatively little effect on measures of central tendency such as the average rate of growth or the median body condition.

1.2 A definition of disease

The definition of disease that will by used in this book is that the term includes "*any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors*" (Wobeser 1997). No distinction will be made between infectious and non-infectious causes of disease because the basic principles of investigation and control are similar for both. However, the term **risk factor**, rather than **causative agent**, will be used when referring to some non-infectious diseases.

Within this broad definition of disease, groups of affected animals with similar features are classified into categories or are said to be affected by a particular *disease* that is identified by a specific name. There is no consistent pattern or rationale for naming diseases, so the current situation represents a hodge-podge of styles:

Name of disease	Derivation of name
Tyzzer's disease	Discoverer (E.E. Tyzzer)
Tularemia	Locale of first description (Tulare County, California)
Bluetongue	Clinical feature
Enzootic ataxia	Clinical and epizootiological features
Avian vacuolar myelinopathy	Pathological feature
Aspergillosis	Causative agent (*Aspergillus* spp.)

In many cases, the name applied to a disease reflects the current understanding of its nature and this name is open to change as the cause or nature of the disease is elucidated. Categories or diseases may be subdivided when it is discovered that several causes produce similar features. For instance, the disease tularemia is now known to be caused by four closely related bacterial species in the genus *Francisella* and types A and B tularemia are recognized. In general, the tendency with time is to define the characteristics of a disease more precisely, and to indicate the causation in the name. For example, a common disease of domestic cattle has gone through a progression of names from red nose, to infectious bovine rhinotracheitis, to bovine herpesvirus I infection. Unfortunately, several names may be applied to a single disease simultaneously, resulting in unnecessary confusion. Thus, a single disease of waterfowl caused by one virus is referred to as duck plague, duck virus enteritis, duck viral enteritis, and anatid herpesvirus infection.

1.3 Disease causation

The study and understanding of the cause and nature of disease have undergone a gradual evolution. Prior to discovery of the identity of specific infectious agents, there was considerable controversy between the alternate hypotheses of the *contagium vivum* (or living contagion theory) and the *miasma* or (bad air theory). The discovery of microbial pathogens silenced this controversy for a period and, at the turn of the past century, both human and veterinary medicine were concerned primarily with identification of specific agents responsible for acute infectious diseases. A set of rules (Koch's postulates) developed for establishing cause-and-effect relationships between infectious agents and disease were generally accepted and widely applied. These stated that the agent:

- must be shown to be present in every case of disease through its isolation in pure culture;
- must not be found in cases of other diseases;
- must be capable of reproducing the disease in experimental animals;
- must be recovered from the host in which experimental disease was produced.

These laboratory-based criteria for judging causal relationships to disease were valuable in defining many diseases of simple etiology, and are still useful in the study of certain diseases such as rabies. However, this one agent-one disease model is not adequate for either the study of many diseases or for the explanation of how most diseases behave in nature. Koch's postulates are particularly inappropriate for many non-infectious diseases, for diseases caused by mixed infections, for diseases in which the predisposing factors are important, for diseases with a carrier state, for diseases caused by opportunistic agents that may or may not cause disease when present, and for many chronic diseases in which the inciting cause has disappeared before the clinical disease becomes evident. Hanson (1969) eloquently outlined the deficiencies of these postulates for the study of wildlife diseases in a presentation entitled "*Koch is dead*" to the Wildlife Disease Association annual meeting.

A more holistic view is necessary for the understanding of most diseases. Jekel et al (1996) proposed three categories that are useful for considering potential agents or factors as the cause of disease:

- If a **sufficient cause** is present the disease will always occur;
- If a **necessary cause** is absent the disease cannot occur;
- A **risk factor** is a characteristic that, if present and active, increases the probability of the disease occurring.

Multiple features of each of the **agent**, the **host** and the **environment** in which the disease is occurring must be considered in evaluating disease causation

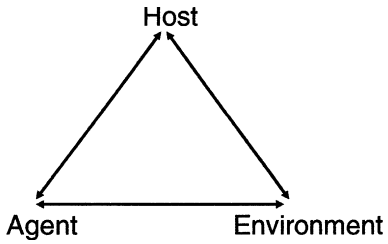

Fig. 1.1 This schematic often is used to symbolize the interactions among agent, host, and other environmental factors that govern the occurrence of disease

(Fig. 1.1). (Consideration of environmental factors recalls the miasma theory of prior times). Even when dealing with a disease caused by a single agent, each of the three major components has a variety of determinants, any of which may influence whether or not overt disease will occur. This simple table presents only a few such variables:

AGENT	HOST	ENVIRONMENT
Strain	Species	Climate
Dose	Genotype	Weather
Route of exposure	Age	Altitude
Duration of exposure	Sex	Other species
	Nutritional status	Population density
	Reproductive status	Air and water quality
	Past exposure	Soil
	Concurrent disease	Human activity
	Immunocompetence	
	Food habits	
	Behavior	

For many diseases, even this agent-host-environment approach may be inadequate and it is more appropriate to consider disease in terms of a **web of causation** in which many factors interact to result in disease. It often is difficult in these situations to classify a factor as being distinctly a feature of the agent, the host, or the environment. Any single factor may be a necessary component but may not be sufficient, in and of itself, to produce disease without the presence of co-factors. The 'lungworm-pneumonia complex' of bighorn sheep (Forrester 1971) provides an excellent example of this type of situation. A wide variety of infectious agents including parasitic nematodes (*Protostrongylus* spp., and less commonly *Muellerius* sp. lungworms), bacteria (*Pasteurella* spp., *Mannheimia haemolytica*, *Arcanobacterium pyogenes*, *Streptococcus* sp., *Staphylococcus* sp., *Neisseria* sp., *Chlamydophila psittaci*, *Mycoplasma* sp.) and viruses (parainfluenza 3 virus, respiratory syncytial virus, bluetongue virus) have been recovered

from sheep dying during outbreaks of pneumonia. It has not been possible to fulfill Koch's postulates completely with any of these agents. Some of the agents are present in healthy as well as in diseased sheep and others have been present in some outbreaks and but not in others, and experimental infection with individual agents either does not result in disease or produces disease that is dissimilar to the natural condition. Many of the agents have been described as predisposing, contributing or opportunistic factors, and none has been identified as **the** cause of the disease. In addition to the infectious agents, many environmental and host risk factors also have been suggested to contribute to the occurrence of this disease. These include overcrowding, interspecific and intraspecific competition, human harassment, poor range quality, contact with domestic livestock, deficiency of trace minerals, above normal rainfall, and inclement weather. Each of these factors is, in turn, influenced by many other factors, so that one could construct a very complex web of causation (Fig. 1.2). Many of the associations in this web are unproven, and will be difficult to prove without experimental manipulation. Even within such a web, it is very tempting to search for a primary cause, and the one agent-one disease concept is still prevalent both among the public and many professionals. (*Pasteurella* spp. and *Mannheimia haemolytica* are the current front-runners among agents considered important in pneumonia in wild sheep). To further complicate the matter, disease complexes such as this often are found, on dissection, to consist of a number of similar but distinct disease entities, each with its own web of causation. This is probably true of pneumonia in sheep in

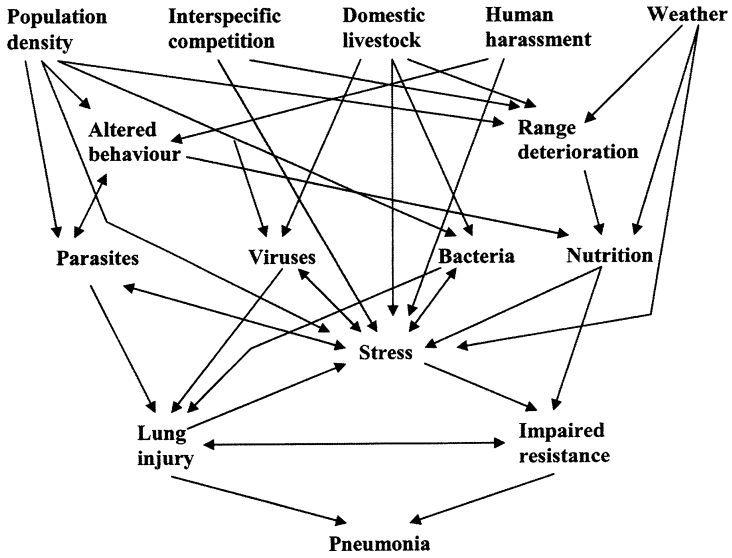

Fig. 1.2 Schematic diagram to illustrate the interrelatedness of various factors that may be associated with, and form a web of causation for, the 'lungworm-pneumonia complex' of wild mountain sheep. Many of these associations are speculative, and the list of factors is likely far from complete

which there appear to be different combinations of viruses, bacteria and other factors in each geographic location with effects on sheep ranging from inapparent infection, through mortality of lambs, to 'all-age' die-offs.

A set of criteria for establishing causation, adapted from Kelsey et al. (1986), reflects the multifactorial nature of most diseases:

1. The hypothesized cause should be distributed in the population in the same manner as the disease.
2. Occurrence of disease should be significantly greater in those exposed to the hypothesized cause than in those not so exposed.
3. Exposure to the hypothesized cause should be more frequent among those with disease than in those without disease, when all other risk factors are constant.
4. Temporally, disease should follow exposure to the hypothesized cause.
5. The greater the dose or length of exposure to the hypothesized cause, the greater the likelihood of occurrence of disease.
6. For some diseases, a spectrum of host responses along a biologic gradient from mild to severe should follow exposure to the hypothesized cause.
7. The association between the hypothesized cause and disease should be evident in various populations studied by different methods.
8. Other explanations for the association should be eliminated.
9. Elimination or modification of exposure to the hypothesized cause should decrease occurrence of the disease.
10. Prevention or modification of the host's response on exposure to the hypothesized cause, e.g., through immunization, should decrease or eliminate disease.
11. Disease should occur more frequently in animals exposed experimentally in an appropriate manner to the hypothesized cause than in control animals not so exposed.
12. All relationships and findings should make biologic sense.

Recognition of the complex interrelationship among various factors allows formulation of hypotheses that can be examined and tested, and points in the web can be identified where control measures might be applied. Jekel et al. (1996) suggested that there are three basic steps in the determination of cause and effect (after putative causes have been identified): (i) investigation of statistical associations between cause and effect, (ii) investigation of the temporal association, and (iii) elimination of all known alternative explanations. These will be explored further in later chapters.

1.4 Disease investigation

The basic reasons for studying any disease are to determine its nature and cause, assess its significance (i.e., to determine the effect of disease on individuals and on the population), and identify methods to prevent, control, or

reduce the disease or its effects. Other reasons for studying disease conditions in wild animals might include curiosity about disease as a biological phenomenon, concern that wild animals have a role in diseases of humans and domestic animals, use of wild animals as monitors or indicators of undesirable changes in the environment, public concern about conditions such as parasites in game animals and highly visible die-offs, concern about the impact of disease on the wild population, and the use of disease to manage pest or problem wildlife.

Disease may be approached through either the study of its course and effects in the individual, or by studying the occurrence, distribution, and effects of the condition in a group or population. These two types of study are often termed **clinical studies** and **epizootiology**, respectively. This book deals primarily with disease in populations but information from clinical studies is required for diagnosing and defining individual diseases. Clinical studies often are required to confirm hypotheses about associations and cause-and-effect relationships.

1.5 Basic epizootiological terms

The words epidemiology and epizootiology often are used interchangeably in discussion of disease in animals. Epidemiology is defined as the study of occurrence of disease in populations and is derived from *epi* = on or upon + *demos* = the populace, and probably dates to the great plagues or epidemics inflicted upon the human populace. Epizootiology has a similar meaning with reference to animals, and will be used in this book. Epizootiology is a quantitative science. The basic methods involve description and characterization of groups of individuals so that quantitative comparisons can be made among groups and so that associations among various factors can be measured. This may involve observation and documentation of naturally-occurring events, such as determining the relative rate of mortality of different age groups during a die-off (**observational epizootiology**), or of studying the effect of some manipulation on the occurrence of a disease (**experimental epizootiology**). A study of the efficacy of a vaccination program for control of rabies in foxes would be an example of the latter type. The objective in epizootiological studies is to collect numerical information that can be applied to the solution of one of the three basic problems of causation, significance, or management.

Description and characterization of disease is done through the use of terms that have a specific and restricted meaning. Unfortunately, many of these terms often are misused. Because a word means what one says it means, some of the terms used most commonly will be discussed here to reduce confusion later.

The general pattern of a disease within a population is characterized by the number of cases that occur over a given time period, relative to the number of cases that would be expected or that would occur normally during that

period. An **enzootic** disease is one that occurs in a population at a regular, predictable, or expected rate. An **epizootic** disease occurs when a disease appears at a time or place where it does not normally occur, or with a frequency substantially greater than that expected for the time period. Thus, an epizootic is said to occur when there is an increase in the number of cases over past experience for a specific population, place and time.

The less precise descriptive terms **outbreak** and **die-off,** which refer to a large number of cases occurring within a short period of time, are not necessarily synonymous with epizootic. For example, sudden explosive outbreaks of botulism occur annually with predictable regularity among waterfowl on some wetlands. Because it occurs on a regular basis, botulism is, by definition, an enzootic disease in these wetlands. Similarly, all males in the population of the Australian dasyurid marsupial *Antechinus stuartii* die abruptly each year (Barker et al. 1978) but this must be regarded as an enzootic event. In contrast, even a single case of a disease may represent an epizootic, if it occurs at a time or place where that disease does not occur normally. Thus, a solitary case of foot-and-mouth disease in a deer, anywhere in North America, certainly would be treated as an epizootic! A single disease may occur in different patterns at different locations, e.g., hemorrhagic disease, a viral disease of deer, is enzootic in white-tailed deer in the southeastern United States but occurs as isolated sporadic epizootics in more northern areas.

Classification of a disease occurrence as either an epizootic or an enzootic event is dependent upon knowledge of its prior occurrence in an area and the classification may change as any new information is gathered. Avian cholera was considered an epizootic disease when it was first discovered among wild geese in Saskatchewan in 1977; however, we know now that the disease occurs at a similar rate each spring within this population and, thus, its status has changed to that of an enzootic disease in this area. Similarly, West Nile virus infection in North America appears to be in the process of changing from an epizootic to an enzootic disease as it becomes established. Packer et al. (1999) used serologic data collected over a 30-year period to classify viral diseases of lions in the Serengeti. Two viruses (feline herpesvirus and feline immunodeficiency virus) occurred enzootically, while four other viruses occurred as discrete epizootics. The terms epizootic and enzootic are based on the frequency of occurrence of cases and not on the severity or duration of the clinical disease in individual animals. Thus, rabies is an enzootic disease in some areas although many animals may be affected and the disease has a short, fatal clinical course. Conversely, a mild, chronic disease may occur in epizootic form.

The most basic quantitative measurement that can be made of a disease is a count of affected individuals. However, such counts may have little value unless they can be put into context. For instance, a count of five striped skunks with rabies has relatively little meaning, except to indicate that rabies is present in the area, unless it can be seen in relation to the group or population from which the animals originated. Finding five rabid skunks would

have much different significance if the animals were found in a sample of ten (i.e., 50% of the sample were affected), than if the five rabid skunks were found in a sample of 1,000 skunks, in which case only 0.5% were affected. A major concern in epizootiology is the identification of suitable population denominators that can be used to convert **counts** into **proportions**. While simple counts are of limited value for making comparisons, proportions can be used in many ways to describe and compare groups. Unfortunately it is often difficult (if not impossible) to identify or count suitable population denominators in wild animals and many studies have resulted only in 'dangling numerators' that are of limited use. If we return to the example of the rabid skunks, the usefulness of proportions versus counts should be obvious. By using proportions, comparisons can be made between areas, years, or age groups:

	County A	County B
Number of rabid skunks	5	5
Number of skunks examined	100	880
	1986	1987
Number of rabid skunks	5	5
Number of skunks examined	10	650
	< 6 months	> 6 months
Number of rabid skunks	5	5
Number of skunks examined	100	1,000

In each of these examples, the raw count of diseased animals was identical in the two subsets of information but the proportion of infected animals was markedly different. Even simple comparisons of this type may provide valuable insights to the disease.

Certain specific proportions or rates are used frequently in epizootiology. The most commonly used of these, **prevalence** and **incidence**, often are misunderstood and misused. **Prevalence describes the frequency of occurrence of disease within a group at a specific point in time,** i.e., prevalence = the number of animals with disease at a specific time divided by the total number of animals in the group at the time. Theoretically, the point in time should be an instantaneous snapshot or cross section of the group. In practice, measurements are usually made over a short period of time, such as one or a few days. Thus, the apparent prevalence of ringworm, a fungal infection, among mule deer killed by hunters in southern Saskatchewan during period of a few days in 1985 was 1.82% (9/494) (Wobeser, unpublished data). (The term "apparent prevalence" is used here because it is unlikely that all of the deer that may have been infected would have been detected by simple visual exam-

ination of the dead animals, thus, the true prevalence of infection was likely higher). Estimating the true prevalence may be important in some situations, such as when trying to assess progress toward eradication of a disease, e.g., see O'Brien et al. (2004). Although the point of measurement for prevalence is usually a period in time, it also may be an event that happens to different individuals at different times. For example, it is correct to calculate the prevalence of congenital anomalies in duck embryos examined on the 20th day of incubation or the prevalence of antibodies to a particular agent among 4-month-old fawns.

Incidence describes the rate of development of a disease within a group during a specified period of time, i.e., incidence = the number of animals developing disease per unit time divided by the number of animals at risk in the group. Incidence refers only to **new** cases that develop during the time period. Animals that had the disease or that were immune to the disease at the beginning of the period are excluded from the denominator, because they were not at risk of developing the disease. Incidence rates are used less frequently than prevalence rates in studies of wildlife, because of the difficulty in following individual animals over a period of time. The most common method used to measure incidence is to examine animals at the start of a time period and then to examine them again at some later date. The incidence rate is calculated from the number of new cases that have developed during the interval. An example of this type of study is the use of sentinel chicken flocks to monitor the amount of arthropod-borne viruses, such as western equine encephalomyelitis virus, occurring in an area. Chickens known to be free of the disease and that do not have antibodies (and, hence, are at risk) are placed in the environment and then monitored at regular intervals for infection or the occurrence of antibodies. The incidence rate (based on the number of new cases detected during the time period) provides an index of the amount of virus activity in the area during the period. This rate can be compared with the incidence rate in other years, and the information can be used to predict the likelihood of an epizootic. A similar technique has been used to measure the incidence of West Nile virus in nestling wild birds. The information can be used to assess the need for management measures, such as vaccination of horses, and measures to reduce human exposure to mosquitoes.

Incidence rates also can be calculated in other ways. For example, the incidence of exposure to a disease in deer during the first 6 months of life could be calculated by measuring antibodies to the agent in blood collected from fawns killed by hunters in the autumn. This approach includes certain verifiable assumptions including that:

- all deer were uninfected and susceptible at birth;
- exposure did not result in the death of deer;
- antibodies are the result of active exposure;
- all infected animals develop antibodies;
- antibody titres persist at measurable levels for at least 6 months.

The rate calculated from such a study would probably underestimate the true rate of exposure because recently exposed animals may not have had an opportunity to develop antibodies and some animals may fail to develop antibodies, even though they were exposed. Despite these deficiencies, the information could be used to make comparisons with similar data from other areas or other years.

In many instances in wildlife disease work, the rates measured are neither true prevalence nor true incidence rates. For example, the proportion of caribou found to be infected with brucellosis among a sample collected throughout an entire year is comprised of the prevalence of the disease at the start of the year, plus the incidence (new cases) throughout the year. In such instances, it is likely more appropriate to report a simple proportion, such as the proportion of infected caribou among the sample collected during the year, rather than using either prevalence or incidence. Qualified terms such as period prevalence rate in which the time period is specified also might be used.

Other rates used less frequently than prevalence and incidence are the morbidity, mortality, and case fatality rates. The morbidity rate is the number sick and mortality rate is the number dying during a unit of time divided by the number in the group during that time. These are analogous to an incidence rate but measure illness and death rather than occurrence. The case fatality rate is the number of individuals dying of a disease divided by the total number with the disease, and can be used as a measure of the severity of the disease. Any of these rates may be applied to subgroups within a population, such as subgroups based on sex or age. For example, the age-specific apparent prevalence of ringworm in the mule deer mentioned earlier was:

Age class (years)	Prevalence of ringworm
0.5	0.6% (1/181)
1.5	2.1% (2/94)
>2.5	2.7% (6/219)

The prevalence of two different factors within a group of animals may be determined and this information may be used to measure the strength of association between the factors. For example, assume that a group of caribou was examined for the presence of carpal bursitis (inflammation of a bursa on the fore-legs) and for antibodies to the bacterium *Brucella suis* biovar 4, which is thought to be associated with bursitis in caribou. The data below show how the relationship of one variable to another can be examined in a simple 2×2 contingency table. In this example, a sample of 400 caribou was examined and divided into four groups:

	Carpal bursitis		
	Present	Absent	Total
Antibodies			
Present	20 (a)	28 (b)	48 (a + b)
Absent	2 (c)	350 (d)	352 (c + d)
Total	22 (a + c)	378 (b + d)	400 (a + b + c + d)

Twenty animals had both bursitis and antibodies, while 350 had neither antibodies nor bursitis. The prevalence of bursitis was 5.5% (22/400) and the prevalence of antibodies to *Brucella* was 12% (48/400). The data can be used to examine the association between bursitis and *Brucella* infection; the first step in investigating a causal relationship suggested by Jekel et al. (1996). The prevalence of bursitis in those animals previously exposed to *Brucella*, as indicated by the presence of antibodies, was 41.7% (20/48), whereas the prevalence of bursitis in those without antibodies was only 0.6% (2/352). One way of measuring the relative strength of association between two factors is through calculation of the **odds ratio** (ad/bc), which is the ratio of disease occurrence between the two groups. If there is no association between the factors, the odds ratio would be 1. In this case the odds ratio is 125, which indicates a strong association between bursitis and exposure to *Brucella*. This does not prove a causal relationship between brucellosis and bursitis but it does indicate an association worth exploring further. This and other methods for examining the strength of the association between a factor and a disease will be discussed further in subsequent chapters.

Many features of disease, such as growth depression, antibody titre, number of parasites, and thickness of eggshells, occur on a quantitative or continuous scale, rather than on a qualitative or yes-no relationship. Such attributes are described in terms of distribution, measures of central tendency (mean, median), and of dispersion (range, variance, standard deviation). Comparison among groups in these instances is by standard statistical methods. (Because of the homogeneity in relative susceptibility within a population, it may be important to consider both the variance as well as measures of central tendency to detect effects on small segments of the population). The severity of expression of disease is often proportional to the level of the causative agent present and, as noted earlier, observation of a dose:effect gradient is generally considered to be evidence for a causal relationship. For example, the thinning of eggshells caused by certain pesticides has been shown to be directly proportional to the concentration of the chemicals within the egg in several species of bird (Blus et al. 1972; Ohlendorf et al. 1978). This type of relationship can be measured by standard methods for determining correlation and regression among the variables. This system of investigation can be extended to the simultaneous collection of information

on a large number of agent, host and environmental variables, and analysis of the resulting data by multi-variate analysis. In this way, the strength of association among many factors to the disease can be measured. Carey et al. (1980) provide an excellent example of this type of study in their characterization of the landscape epizootiology of Colorado tick fever.

Highly sophisticated techniques are available for the collection and analysis of epizootiologic data; however, the investigator must always be concerned about: (i) how representative the samples are of the population, (ii) the difference between statistical and biological significance, and (iii) the need to ensure that the methods and findings have biological relevance. As stated by Friedman (1980), "*no method of analysis, no matter how mathematically sophisticated, will substitute for careful evaluation of data based on good scientific judgment and knowledge of the disease process being studied*".

1.6 Summary

- Disease in wildlife is often of multifactorial causation, and the effects of many diseases on wild animals are understood poorly.
- Investigation of disease is undertaken for three basic reasons: to determine its cause, to determine its significance, or if justified, to identify methods for management.
- Disease may be studied in the individual animal or in the population; a combination of methods is usually required.
- Epizootiology involves the description and characterization of variables associated with disease in groups of animals and the comparison of factors among groups.
- Qualitative aspects of disease, such as the presence or absence of some factor, are evaluated by determining the proportion of the group affected, or the rate of occurrence. These rates form the basis for comparison among groups.
- Many features associated with disease occur on a continuous scale. These are described in terms of the distribution of occurrence and by measures such as mean and standard deviation. Association among factors is investigated by techniques for measuring correlation.
- Results of investigations must make biological as well as mathematical sense.

2 Special problems in working with free-living animals

> *"Usually, insufficient attention is paid to the infectious and parasitic diseases of wildlife until some outbreak of disease, no matter whether in wildlife or domestic animals, when the importance of disease or infestation of wildlife is often overestimated."*
>
> (Jansen 1964)

Although the same basic techniques are used for the study and management of disease in wild animals, domestic livestock, and humans, the wildlife specialist encounters difficulties that are unimportant or that can be controlled, literally or statistically, in studies of the other two groups. Most of these difficulties are a result of the 'wildness' of the subject animals. The word **wild** has many meanings, including "*growing without the care of man*", "*unaffected by civilization*", "*of great violence or intensity*", "*undisciplined*" and "*extravagant or fantastic*". No wild animal is unaffected by civilization, since all inhabitants of the globe share effects, such as chlorinated hydrocarbon residues and global warming, but most wild animals grow without (and some grow despite) the 'care' of humans. Most of the other definitions are applicable to free-ranging species. Disease is notoriously hard to detect, even in humans and domestic animals. Disease in wildlife has often been compared to an iceberg with only a tiny fraction or tip of the total mass being visible at any time. Part of this phenomenon is because very few people are looking for, and even fewer are reporting, disease when it does occur. However, the covert nature of disease, and particularly its quantitative aspects, makes disease inherently more elusive in wild animals than in either livestock or humans. The wildlife worker has a much greater difficulty finding diseased individuals than does either the physician or the veterinarian, and one is seldom able to count wild populations in the way that cattle in a pen or children in a school can be counted.

Arrival at an understanding of any disease is a slow, gradual process analogous to unwinding a ball of string comprised of many short lengths. Each bit of string removed, or fact discovered, brings one nearer to the core, so long as the fact is recorded and available to the next researcher. The study of disease in wild animals is a new science and there are relatively few scientists in the field, so that many of the facts taken for granted about humans and domestic animals remain to be discovered. The extravagant and fantastic

nature of wild species and their undisciplined response to various procedures create unique problems for those interested in disease, as does the relationship that exists between the public and wildlife.

2.1 Problems in detecting diseased animals

The most basic quantitative measure of disease in a group is an enumeration of affected individuals. In human and veterinary medicine, the detection of sick individuals depends upon the severity of clinical signs, the willingness or desire of the patient (or the owner) to seek and allow examination, the diagnostic personnel and facilities available, and the skill of the diagnostician. All of these factors also apply to the study of disease in wild animals but, in addition, disease detection is further complicated in this group by the difficulty of finding sick animals. In a few situations, disease may make affected wild animals overly available and this may cause problems of bias in samples. For example, rabies might make animals prone to be killed by automobiles, so that a sample of road-killed skunks may not be indicative of the actual prevalence of that disease in the population of an area. Similarly, Bellrose (1959) found that ducks that had ingested lead shot were more likely to be killed by hunters than were normal ducks. Hence, lead-exposed birds are likely to be over-represented within the hunters' bag. However, these examples are exceptional cases and, in most instances, sick animals become less rather than more readily available to the investigator. This is because of the restricted travel, secretive behavior, and increased susceptibility to predators that occur among sick animals. Predators and scavengers are usually in direct competition for specimens with the researcher, (but they may be cooperators in disease-management programs based on population reduction or carcass disposal).

When wild animals die, their carcasses are "*quickly assimilated into the environment*" (Stutzenbaker et al. 1986). The investigator who is measuring mortality based on counts of dead animals must consider two factors: (i) the proportion of the carcasses present that is detected, i.e., the efficiency of the search technique, and (ii) the rate of disappearance of carcasses as a result of decay and scavenging. Many descriptions of disease outbreaks contain estimates of the relationship between the number of animals found sick and/or dead, and the total number that died. For example, Hoff et al. (1973) suggested that the recovery rate of carcasses during an epizootic among deer in North Dakota was "*not more than 10%*" and then multiplied the number of deer found dead by a correction factor of ten to obtain an estimate of total mortality. However, these authors did not provide information on how the estimate of a 10% recovery rate was determined or of its accuracy. Other investigators have attempted a more quantitative approach to deal with this problem. Following a similar epizootic among deer in Montana, Swenson (1979) searched a large area and found 34 carcasses. He then marked the 14 carcasses found on a portion of the area and asked hunters to record and mark all

carcasses that they encountered on this portion. (This technique is a form of the classical mark-recapture method used widely by biologists for estimating animal numbers that will be discussed in Chap. 5). Hunters found 51 carcasses, including the 14 marked ones, at the area. Swenson used the ratio 14/51 to calculate that his search had located (at *maximum*) 27% of the carcasses present on the area. He adjusted the count on the overall area by a correction factor of 3.6 (51/14) to estimate that a *minimum* of 124 deer died at the entire area.

Some researchers have examined the efficiency of carcass searches experimentally (Robinette et al. 1954; Anderson 1978; Humberg et al. 1986; Stutzenbaker et al. 1986; Ward et al. 2006). Stutzenbaker et al. (1986) studied the effectiveness of search crews in locating dead ducks in a shallow Texas wetland. One hundred banded duck carcasses were distributed in a 40-ha marsh, with 50 of the birds placed in "*typical escape cover*" to simulate birds dying of chronic lead poisoning and 50 placed in "*completely exposed positions atop vegetation*" to simulate ducks that died of rapidly fatal avian cholera. Within 30 min of placing the carcasses, eight searchers (unaware of the carcass placement) were asked to search the area and to collect sick and dead birds. The searchers failed to find any of the birds placed in cover and found only six (12%) of the "*highly visible*" carcasses. The authors concluded that "*lack of carcasses recovered during intensive searches does not rule out extensive waterfowl mortality. Thus, casual searches would result almost invariably in negative findings even though large numbers of birds actually died*." We found that the success in finding carcasses of ducks that had died of botulism during carcass searches is highly variable and as few as 7% of the marked carcasses may be recovered on large wetlands.

The rate of disappearance of carcasses also has been studied under a variety of circumstances (Wobeser et al. 1979b; Humberg et al. 1986; Stutzenbaker et al. 1986; Pain 1991; Wobeser and Wobeser 1992; Cook et al. 2004; Ward et al. 2006). Although the results of these studies were somewhat variable, it appears that about 50% or more of duck to goose-sized carcasses disappear within 4 days, and that 75% of passerine bird carcasses may be removed within the first day. Given this information, it is not surprising that smaller animals such as passerine birds, rodents, and neonatal ungulates, are seldom found dead. An exception to this rapid rate of carcass disappearance may occur when a large number of individuals die within a short period of time in a small area. The resulting plethora of carcasses appears to temporarily overload the normal removal mechanisms (Linz et al. 1991) and individual cases may persist for an extended period. We have found this to be true of duck carcasses marked and observed during a botulism outbreak. In one such situation, only 1 of 42 duck carcasses was disturbed by scavengers during the first 4 days after death (Cliplef and Wobeser 1993). If carcasses are removed rapidly by scavengers, it is obvious that mortality surveys based on regular, e.g., weekly, counts of dead animals contain a very significant underestimation bias.

Any detailed study of diseases that is based on the recovery of sick or dead animals should address these problems and attempt to measure the efficiency of the data collection methods used. Raw counts without some adjustment

cannot be used to calculate **absolute** morbidity or mortality, but could be used to monitor **relative** changes, as between years, providing that other factors remain constant.

2.2 Problems in determining numbers and identifying individuals

Epizootiology is a quantitative and comparative science. In human populations, at least in developed countries, and to a lesser extent in domestic animals, population parameters are obtained by **census.** This implies an actual count of all individuals, together with collection of details such as the sex and age composition of the population. It is seldom possible to census wild animals completely, except under unusual circumstances. Such circumstances may occur when a small number of conspicuous animals are located in an isolated area, e.g., the wolves and moose of Isle Royale, Michigan (Mech 1966), or when a highly visible species congregates in a small area, e.g., the mid-continent population of sandhill cranes on the Platte River in Nebraska (Buller 1979). In other situations, the person interested in wild animals usually must make do with an **estimate** of the number of animals in the population. An estimate may mean either a guess (an opinion without sufficient evidence) or a term referring to an average and its range of values as determined by a set of rules (statistics) (Davis and Winstead 1980). Unfortunately, many of the estimates used in wildlife disease work have been of the guess-type and wildlife biologists often must deal with population estimates that are of unknown reliability or that have very wide confidence limits.

Statistical estimates are obtained by sampling. The techniques include methods such as counting animals on a portion of the total area with quadrat or transect surveys, measuring some type of ratio of abundance such as the number of pheasants heard crowing/hour or the number of birds seen/km of road, or by using an index to abundance in which some object associated with the animal is counted rather than counting the animals, e.g., counting muskrat houses rather than muskrats. Methods for measuring population parameters will be discussed in detail in Chap. 4. At this point it is important to recognize the difference between a census and an estimate as well as to realize that even 'good' estimates of population size in wild animals often have wide confidence limits. The latter factor becomes problematic when trying to assess the impact of disease on a population or to assess the effectiveness of some management. For instance, if the best possible estimate of population size has confidence limits ±30% of the mean, it will be difficult (or impossible) to detect the impact of even major disease events on the population. Harding et al. (2005) provide an example of the extent of sampling that is needed to detect changes as great as a 60% decline in the population of some species.

Humans have names, social security numbers, and other features that identify us as individuals. Domestic animals are usually identifiable by tags, brands, tattoos, or by linking them to their owner. In contrast, wild animals are anonymous, except for a tiny proportion of the population that may have been marked by biologists. This means that one powerful tool commonly used for the study of disease in humans and domestic animals is impractical for the study of many free-ranging species. In human and veterinary medicine, individuals often can be traced backward in time to determine if they have been exposed to a certain factor, or forward in time to determine the outcome of exposure to a factor. Thus, if we are interested in the relationship between smoking and heart disease, we might determine, through questioning, the smoking history of a group of cardiac patients. Alternatively, we might follow a group of smokers for several years to compare the incidence of cardiac disease in this group relative to that in a similar group of nonsmokers. Such retrospective trace-backs and prospective studies are seldom possible in wildlife. For example, a mallard found dead in a pond in Saskatchewan in early spring might have just arrived from a wintering area located anywhere between Florida and California. There are no distinguishing features or marks on the bird to indicate its recent past and there is no simple way to trace back to see if it may have been exposed to a pesticide in Arkansas, an avian cholera outbreak in Nebraska, or duck plague virus in California. In such cases, all one can do is perform specific analyses to look for residues or antibodies to each of the possibilities. Looking for residues or antibodies is like looking at animal tracks in the mud, the tracks tell you that something has passed but only an expert tracker can estimate when the event occurred. The results of analyses may indicate past exposure but will not tell when or where the exposure occurred. Similarly, it would not be possible, without a massive marking and monitoring program, to follow the fortunes of a group of birds that were exposed to a particular disease agent at a specific site. In wild species, one seldom is able to follow the clinical progression of naturally occurring disease in an individual, and most diseased individuals are not detected to be sick until they are in extremis or dead. A method that can be used to follow animals forward in time is through the use of radiotelemetry. For instance, Moriarty et al. (2000) followed 247 radio-marked adult rabbits for a year in Australia and found that the overall mortality rate of 82% was comprised primarily of deaths caused by predation (44%), rabbit hemorrhagic disease (16%), and myxomatosis (9%).

In general, humans and domestic animals are rather sedentary, while many wild animals are highly mobile. When dealing with sedentary species, the investigator can be reasonably confident that he is looking at approximately the same population from week to week. This is not the case with mobile (and especially migratory) wildlife. During a study of avian cholera in geese in Saskatchewan, we measured the size and species composition of the goose population on a study area by conducting weekly aerial surveys (Wobeser et al. 1979b). We were able to determine the approximate age

distribution within some species by counting the number of juvenile and adult birds within flocks. Thus, we were able to estimate the overall population and its approximate composition each week; however, we could not tell which individual birds were present from one week to the next. It is obviously very important to collect this type of information if the length of exposure to some factor is important in a disease. In this example, and in many other situations involving mobile wildlife, periodic estimates of population are analogous to a series of still photographs, taken from above, of a revolving door in a busy building. The number of individuals within the doors in each photograph can be counted, but it is unclear whether the faceless individuals are going round and around, i.e., a sedentary population, or if new persons are continually passing through in one or both directions. Lehnen and Krementz (2005) used a sample of radio-marked birds to estimate the average time that pectoral sandpipers spent on a staging area and the degree of turnover in the population with time, and used this information to assist in estimating the total number of sandpipers passing through the site during migration.

The ability to distinguish between residents and transients is usually critical in disease investigation. This is particularly true when trying to evaluate the effects of short-lived phenomena, such as a pesticide application. For example, one method for evaluating the effect of a pesticide spray program on birds might be to count birds in the area immediately before and then again a few days after spraying. If the population size remained approximately constant before and after spraying, this might be interpreted to mean that the spray had no effect. However, the same findings would occur if some, or all, of the population present at the time of spraying died but were replaced by new birds not exposed to the toxin. In such a circumstance, it would be critical to be able to differentiate between residents and transients. It might be necessary to capture and mark a large number of the birds on the area prior to the spray application, and then do a recapture program to confirm that these individuals were still present after spraying, in order to identify population turnover.

Another difficulty that may be encountered when working with mobile wild species is that disease may occur only during a portion of the year when the animals are inaccessible or difficult to observe. For instance, heavy infestations with the flea *Ceratophyllus vagabundus* occur on lesser snow and Ross' geese while they are nesting in the arctic (V. Harriman, personal communication) but I have never observed a flea on any of the many Ross' and snow geese that I have examined during spring and autumn migration. Similarly, it is difficult to evaluate the effects of oil pollution on seabirds, because the birds can only be counted on breeding areas, while oil spills usually occur in remote wintering areas among birds dispersed over vast areas (Votier et al. 2005). Population dynamics of a migratory species may be influenced by factors encountered at a staging area that is visited for only a short period of time (Schaub et al. 2005); if the factor is a disease agent and it is not evaluated at that site and time, its effect will not be detected.