Pathology

(A. Disease

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INTRODUCTION

This part of Chapter 17 is concerned with infectious and non-infectious factors that adversely affect the health, well-being and survival of individual birds of prey in the wild or in captivity, and which may influence the conservation status of species in the wild. Toxicology, which is mentioned briefly, is covered primarily in Chapter 18. There are important links between material in this chapter and other aspects of raptor biology that relate to health, including food habits (Chapter 8), reproduction and productivity (Chapter 11), behavior (Chapter 7), physiology (Chapter 16), energetics (Chapter 15) and rehabilitation (Chapter 23). Although ectoparasites and endoparasites are covered elsewhere in Chapter 17, when appropriate, they are mentioned here as well.

I first differentiate "health" and "disease" and define several additional important terms.

Health is a positive concept that is defined by the World Health Organization in relation to humans as "*A state of complete physical, mental and social wellbeing, not merely the absence of disease or infirmity.*" *Disease* (from Old English *dis* = lack of; and *ease*) is taken to mean any impairment of normal physiological function that affects all or part of an organism. As such,

disease can be due to a range of factors, not just infections with pathogens. The causes of disease can be either infectious, including viral infections and parasite infestations, or non-infectious, including injuries and changes caused by trauma, poisons, genetic factors, or environmental stressors. The causes of disease often are multifactorial. For example, raptors that have been nutritionally deprived (inanition, starvation) more readily succumb to the fungal infection, aspergillosis, than otherwise (Cooper 2002). In this instance, the latter is the proximate (i.e., immediate) cause of death, while the former is the ultimate (i.e., predisposing) cause (Newton 1981). Here, I follow the terminology that is favored by ecologists, rather than medical personnel, in that macroparasites include metazoan organisms, such as mites and worms, whereas microparasites include single-celled organisms, such as bacteria and protozoa.

The diagnosis (detection and recognition) and treatment of disease in birds of prey is primarily the responsibility of the veterinarian but, as will be shown repeatedly in this chapter, those from other disciplines, ranging from anatomists and biochemists to DNA technologists and zoologists, also can and do contribute to this work. Monitoring of health of raptors is different from diagnosis. Monitoring of health implies "surveillance of a group or population of birds," and the raptors that are being watched often appear normal. The aim of monitoring in such cases is to compile a health profile of such birds, including understanding which bacteria they carry, whether they have antibodies to certain organisms, their body-condition score, the state of the plumage, etc. The techniques employed in monitoring health often are similar to those used for disease diagnosis. However, the best results are obtained if avian biologists and other non-medical professionals are an integral part of the team (Cooper 1993a).

The two decades that have elapsed since this chapter first appeared in Giron Pendleton et al. (1987) have seen enormous advances in our understanding of the biology of birds of prey and of those diseases that may either cause disease (morbidity) or result in death, and the importance of routine health-monitoring has been widely promoted and put into practice (Cooper 1989).

Health monitoring, essentially, is an early-warning system that can either help to confirm that a population of raptors is free of significant diseases or pathogens or, if these are present, help to ensure that appropriate action is taken without delay. Health monitoring of captive birds of prey is now standard practice in zoos and other establishments, and, increasingly, is the norm in studies on free-living raptors, especially when changes in population numbers or in distribution have been observed or are suspected (Cooper 2002).

The main causes of death and decline in free-living raptors often include environmental factors such as habitat destruction, human persecution, inadvertent human-related injury and poisoning, most of which are well studied in raptors (Newton 1990, Zalles and Bildstein 2000). In contrast, infectious disease as a mortality factor in birds of prey has proved difficult to evaluate, despite the best efforts of various biologists and veterinarians. Important early thinking about the part that might be played by infectious agents in free-living raptors was summarized in Newton (1979) and updated by the same author in 2002 (Newton 2002). Newton discusses the possible impact of infectious agents on raptors and draws attention to the important epidemiological difference between population-dependent and population-independent diseases.

There is increasing evidence from research on other species that when a population of birds becomes isolated and falls below a certain level, infectious (including parasitic) diseases may become relevant factors in demise or survivorship. The effect of infectious disease is likely to be more significant if there is a high inbreeding-coefficient, which can increase susceptibility among individuals. The decline in number of some of the world's birds, and the tendency for many of them to be confined to small islands of suitable habitat, suggests that infectious disease will assume a more pivotal role in the future. Birds of prey occupy a key position at the top of many food chains, and as a result are particularly vulnerable to environmental build-up of infectious (including parasitic) organisms. Small populations appear to be particularly at risk.

Recently, "wildlife-disease ecology" has evolved as a subject in its own right (Hudson et al. 2002). This has been prompted in part by the recognition of new, emerging infections of domestic livestock and humans, some of them with wild animal reservoirs, and by concerns about the possible adverse effects of micro- and macroparasites on free-living vertebrates. Understanding the dynamics of such diseases often entails the use of mathematical modeling as well as field studies, and, as such, involves scientists from many different backgrounds. As a result, a better understanding of host-parasite relations in wild animal populations is unfolding. This new research is likely to help assess the muchdebated role of various organisms in the biology of freeliving raptors.

Some people still question the value of health studies on free-living raptors, arguing that other, mainly non-infectious, factors warrant greater attention. Although debatable, the situation is unequivocal for captive birds of prey. Under such circumstances, infectious disease is recognized as presenting a real challenge. Prompt detection is essential and is the focus of any properly formulated health-monitoring program. For many reasons it is desirable that captive raptors remain free of disease. Perhaps even more important is that birds destined for release into the wild are monitored for infectious disease, both to minimize the chances of their disseminating pathogens in their new environment, and to protect them from succumbing to novel organisms that they may encounter there. Such pre-release and pre-translocation health monitoring, or screening, is recommended by the IUCN Reintroductions and Veterinary Specialist Groups (see, for example, Woodford 2001), and is now a standard feature of many conservation programs globally.

Below I discuss the requirements and techniques for investigating diseases and for monitoring free-living and captive birds of prey as part of so-called health studies.

HEALTH-STUDY REQUIREMENTS

Prerequisites for effective health studies include (1) properly trained personnel, (2) appropriate laboratory and field equipment, and (3) effective interdisciplinary collaboration. Each is discussed and commented upon in turn.

The staff and equipment required for health studies

depend upon the degree of investigation planned. For basic health-monitoring studies, where only a representative number of birds are to be examined or a limited series of tests is to be performed ("screening"), a small team and minimal equipment usually are adequate. More extensive and intensive studies, however, usually require specially trained staff and an appropriately equipped laboratory. Interdisciplinary links are especially important in the field, but also are useful in laboratory investigations. It is unlikely that one person or facility will be able to undertake all the tests and analyses required, and some material may need to be sent elsewhere for toxicological analysis or molecular studies, for example.

Personnel

As a general rule, a veterinarian should coordinate clinical or pathological studies, since they will have broad training in animal disease, including a working knowledge of diagnostic and investigative techniques. There also may be legal implications, especially if a diagnosis is being made or if infectious agents are being handled that may present a threat to domestic livestock or human health (see below). If a veterinarian is unavailable in person, although possibly contactable for advice by telephone or e-mail, the biologist should carry out the work alone or with limited assistance. In such cases recruiting individuals who have a background of working in veterinary or medical laboratory technology is recommended, as such people are likely to have knowledge and understanding of appropriate skills in bacteriology, parasitology, and histopathology.

Researchers who regularly conduct health studies without veterinary guidance should be trained to do so. It is preferable to master a limited number of procedures rather than endeavoring to cope with all contingencies. Quality control should be practiced by periodically submitting material to other institutions for independent assessment to check and verify the work.

Laboratory and Field Equipment

Laboratory resources are essential for all health studies on raptors, whether these constitute disease investigations or health monitoring. There is much to be gained if the facilities are part of a larger complex, such as a university department or a veterinary investigation center, as the latter usually provide a range of other disciplines and personnel. If access to a permanent laboratory is not possible (i.e., when working in isolated sites), laboratory tests may have to be performed in the field. Many clinical kits that can be readily transported and used effectively in difficult terrain, and away from electricity and running water are described in Cooper and Samour (1987). Basic tests can be carried out in the field using equipment and reagents in the kit, whereas others may require material to be transported to a more specialized or better-equipped laboratory.

Whenever and wherever investigations are performed, attention must be paid to the safety of staff and onlookers (see Legal Aspects).

Effective Collaboration with Others

It is important that all those involved in health studies work as a team (Cooper 1993a). From the outset, the raptor biologist should be aware that there are others in disparate disciplines who are likely to provide advice or support. Within a given country, state, or province such collaboration usually is not difficult, but suspicions and jealousy, especially regarding ownership and funding, are possible when things become more regional or international. Researchers should be alert and sensitive to this possibility. Despite closer collaborations among raptor biologists and others recently (Cooper 1993a), a properly coordinated international system for the investigation of morbidity and mortality in birds of prey does not exist (Cooper 1983, 1989, 2002).

TECHNIQUES

Below, I outline some of the methods used to carry out health studies and to sample birds of prey. Details of laboratory and necropsy procedures are given later.

Clinical Methods

Capture techniques are discussed in Chapter 12. The sampling of raptors as part of rehabilitation work is covered in Chapter 23.

Clinical examination and sampling both are part of diagnostic work and health monitoring. This work must be conducted professionally, proficiently, and with a minimum amount of discomfort, pain or stress to the bird. Properly formulated protocols are essential. Detailed information on clinical procedures can be found in several recent texts on raptor medicine and management. Redig (2003) provides an excellent catalog of the veterinary considerations when working with falconiforms or, for that matter, strigiforms, and refers readers who require further information to five authoritative works, including Heidenreich (1997), Lumeij et al. (2000), Redig and Ackermann (2000), Samour (2000), and Cooper (2002).

The principles of clinical investigation include the following sequential stages: (1) history (environmental for free-ranging birds; management for captive birds), (2) observation, (3) clinical examination, (4) taking samples for laboratory investigation, (5) results and diagnosis, and (6) treatment and action. A suggested record sheet for health-monitoring work is in Appendix 1.

Laboratory Investigations

Laboratory investigations are an important part of clinical work, post-mortem examination (see below), and the analysis of environmental samples. Examples of laboratory investigations are depicted in Fig. 1.

Toxicology and chemical analysis are covered in Chapter 18, and are not discussed here. That said, pathologists should work closely with toxicologists and ensure that suitable samples are taken for analysis or stored for later reference. Likewise, carcasses of birds submitted specifically for toxicological examination (e.g., for pesticide analysis) also should be made available for detailed gross and histopathological examination and microbiological studies. Factors other than chemical toxins, including micro- and macroparasites, or underlying renal or hepatic disease, also should be investigated. Other laboratory investigations are discussed and tabulated later in this chapter.



Figure 1. Sample from live bird, dead bird, or the environment.

Special Investigations

Although standard procedures outlined above are applicable to most health studies on raptors, additional laboratory investigations, including microbiological and parasitological monitoring of nests, nest-boxes, aviaries, breeding pens, and incubators, also may prove valuable. Swabs can be taken from such sites and cultured for bacteria and fungi. Food items, likewise, can undergo microbiological or toxicological analysis or both. Ventilation in breeding pens and aviaries can be assessed by smoke tests, and its efficacy calculated by the use of bacteriological "settle plates," or other specific air-sampling methods (Cooper 2002). The laboratory examination of regurgitated pellets is a special feature of raptor health studies that is discussed below.

The Post-mortem or Necropsy Examination

Preparation for a post-mortem examination is all-important. The necessary steps can be summarized as follows:

- Decide why the necropsy is to be carried out. The various categories of examination, each with different objectives, are summarized in Table 1.
- Check that appropriate facilities and equipment are available, including protective clothing and measures aimed at reducing the risk of spread of infectious disease to humans or other animals (see below).
- Be sure that the person carrying out the postmortem examination is sufficiently knowledgeable about the techniques and precautions that are necessary.
- Be familiar with the normal anatomy of the species (cf. King and McLelland 1984, Har-court-Brown 2000) as well as its general biology and natural history (Cooper 2003a).

Health and safety. Raptors can present hazards to those who work with them. These include physical dangers when trapping birds on cliffs or retrieving carcasses from marshes or other wetlands, and chemical dangers due to contact with toxic or carcinogenic agents such as formaldehyde. For the purposes of this chapter however, the potential threat of zoonoses, or diseases and infections that are naturally transmissible between vertebrates and humans, is particularly relevant. A review of zoonotic infections that might be acquired from birds, including raptors, was produced some years

Table 1. Categories of post-mortem examination of raptors.

Purpose	Category	Comment
To determine the cause of death.	Diagnostic	Routine diagnostic techniques are followed.
To ascertain the cause of ill-health (not necessarily the cause of death).	Diagnostic-health monitoring	Usually routine, but detailed examinations and laboratory tests may be needed to detect non-lethal changes.
To provide background information on supposedly normal birds on the presence or absence of lesions, parasites, or of other factors, such as fat reserves or carcass composition.	Health monitoring	As above.
To provide information for a legal case or similar investigation, including determining the circumstances of death or the possibility that the bird suffered pain or distress while it was alive.	Forensic-legal	Usually very different from the categories above. The approach depends upon the questions being asked by police or enforcement bodies who requested the necropsy. There must be a proper "chain of custody/ evidence." All material and wrappings should be retained until the case is closed (Cooper and Cooper 2007).
For research purposes, such as collection of tissue samples or studies on organ weight.	Investigative	Depends upon the requirements of the research worker.

ago (Cooper 1990). A number of publications have followed on the heels of new hazards, including West Nile virus. Palmer et al. (1998) provides a useful general reference to zoonoses, including information on both animals and humans.

It is both useful and legally astute for researchers to have an up-to-date list of zoonoses that may be contracted from birds. Infectious agents that once were considered unimportant in humans now are recognized as being potentially pathogenic. Many of these "opportunistic" species take advantage of a debilitated host; in particular, an individual that is immunosuppressed as a result of another infectious disease (e.g., HIV-AIDS, malaria, etc.), malnutrition or on account of medication that is reducing the immune response. It is prudent to assume that any raptor might be a source of organisms that are pathogenic to humans. If this precautionary approach is followed and appropriate safeguards taken, the risks involved in carrying out an examination of a live or dead bird are minimized.

The specific precautions used to restrict the spread of zoonotic infections depend upon the circumstances. In some countries national health and safety legislation may require the employer of those studying wild birds (including handling, post-mortem examinations or sample-taking) to compile a "risk assessment" before the work commences. The researcher, veterinarian, or technician will need to follow prescribed rules and take appropriate precautions. In some countries rules may not exist or may be poorly enforced. Nevertheless, researchers have a responsibility to protect colleagues and assistants, and it is wise to compile a code of practice aimed at minimizing the risk of infection (Cooper 1996).

Necropsy technique. Many methods have been advocated for the post-mortem examination of birds. Some have been devised by veterinarians, usually for the diagnosis of specific diseases (Wobeser 1981, Hunter 1989, Cooper 1993b, 2002, 2004). Others have been devised by ornithologists interested in wild bird mortality or those needing to obtain samples for research (van Riper and van Riper 1980). A basic technique for those working in the field, especially in areas where access to professional advice is limited, is detailed in Cooper (1983). Specific guidance for the necropsy of birds of prey is provided in Cooper (2002).

Necropsy methods should be efficient and reproducible. A post-mortem examination is not simply a matter of "opening up the body." It is a structured operation that involves both external and internal observations and, usually, detailed investigations of organs and tissues. Young birds and embryos require a different approach (Cooper 2004b and below).

A comprehensive necropsy, which encompasses features of both "diagnostic" and "health-monitoring" investigations, including a range of tests and analyses, in addition to collection of biometric data (see Appendix 2), can be time-consuming. Detailed and exhaustive work is vital when rare or threatened species of raptors are involved or deaths have occurred under unusual circumstances. Under more typical circumstances, when time is at a premium and common species are involved, lengthy and detailed investigations of every bird may not be feasible. At such times the abbreviated postmortem protocol outlined below can be followed, coupled with the appropriate storage of material for subsequent studies:

■ Upon receipt of the specimen, record the history and give the bird a unique reference number. This not only is good practice, but is an essential precaution (to facilitate chain of custody/evidence) if legal action is underway or likely to occur (Cooper and Cooper 2007).

• Examine the bird externally (including beak, buccal cavity, auditory canal, preen gland, and cloaca). Record (and quantify) any parasites, lesions, or abnormalities. Comment on plumage and molt using standard ornithological protocols.

■ Weigh the bird. Record standard measurements. The body mass of a bird is of limited value without measurement of its linear dimensions (i.e., wing chord [carpus], tarsus, culmen, combined head and bill, and sternum). The body mass is the most important and should form part of every examination.

• Dissect (open) the bird from the ventral surface by lifting or removing the entire sternum. Examine superficial internal organs. Record any lesions or abnormalities.

■ Remove and set aside in clean (preferably sterile) containers, the heart, liver and gastrointestinal tract, ligating the esophagus and rectum to prevent the spillage of their contents. Examine deeper internal organs. Note any lesions or abnormalities.

■ Fix in 10% formalin small portions of the lung, liver, and kidney, and any organ or tissue that appears to be abnormal (enlarged, unusual color, containing distinct lesions, etc.).

• Open the proventriculus, gizzard, and portions of intestine. Search with the naked eye and a hand lens for food, other material (e.g., pellets), parasites, or lesions. Examination is facilitated if the material is placed in a Petri dish together with a little saline, and illuminated from below. Save any interesting contents or parasites and make an effort to quantify them, for example, by estimating the proportion of the intestine examined and counting the number seen.

■ After examination, freeze and save the bird's carcass, (or, if more than one bird is available, some frozen and others fixed in formalin) until a decision can be made as to further tests that may need to be performed (see below).

■ Record how and where the body and samples are saved, and include a reminder that they may need to be processed or discarded at a later date.

Appropriate equipment, including a scalpel with blade, scissors, and two pairs of forceps, must be used when conducting the examination. Small ophthalmological instruments may be needed when necropsying nestlings of small raptors, whereas larger, heavy-duty instruments may prove more serviceable for large raptors, such as eagles. Rat-toothed forceps are ideal for grasping tissues during dissection, but can damage samples destined for the histology laboratory. A hand lens or dissecting loupe is invaluable for the investigation of small birds and detecting tiny lesions.

Key features of any post-mortem examination include (1) recording all that is seen or done, (2) taking of samples, and (3) retaining material for subsequent study. The prime objective of any person who is carrying out a post-mortem examination, regardless of training and experience, is to observe and to record. There is an inherent danger in attempting to interpret findings during the post-mortem examination. Something that may appear significant initially, such as damage to a pectoral muscle or pallor of the liver, subsequently may prove to be of little consequence as other findings cast a different light on the case. Bacteriological examination, which typically does not yield results for 3 to 4 days, may reveal that a bird that died with an injured muscle or pale liver, actually died from an overwhelming bacterial infection. Thus, it is prudent to reserve judgment until all tests are complete. If a provisional diagnosis is essential, this should be issued with the caveat that it is tentative, and may be modified pending further results. Many investigations of raptor mortality have been compromised by premature judgments based on inadequate information.

The assessment of "condition," although controversial, is considered an important index in studies on survival and reproductive success. Methods of assessing condition in birds include: ■ Relating body mass to linear measurements (see above). Unwrapped carcasses undergo gradual evaporation, therefore weight loss should be taken into account.

• Assessing and scoring the amount of fat, both subcutaneous and internal.

Measuring muscle (especially pectoral) size, both macroscopically and histologically.

■ Taking whole-body measurements using, for example, the TOBEC system (Samour 2000).

All of these methods have their own devotees. Which is used depends upon the protocol being followed and the facilities available. However, it is important that some assessment of condition be made in order to relate findings from one bird to another. Thus, measurements of carpus must be routine, as should calculations of body mass. A scoring system should be devised and applied to parameters such as the quantity of fat that is visible or the size of pectoral muscles.

Space does not permit detailed discussion of all systems, but mention is made of the reproductive tract because of its importance in assessing and measuring breeding success (Newton 1998). Careful examination of the genitals is essential. Sexing a dead raptor is generally not difficult. However, if a bird is immature or not yet in breeding condition the gonads may be difficult to see. In some instances, post-mortem change (autolysis) can make detection impossible. The use of a hand lens and strong reflected light often helps, but if this also fails, a portion of the kidney and the presumed gonad can be examined histologically to determine the sex. Notes always should be taken of the appearance of the ovary or testes. In the falconiforms, the presence or absence of a vestigial right ovary should be recorded as part of developing a biomedical database. The color of the testes should be noted as they are sometimes pigmented. Whenever possible, and always when a series of birds is being examined and compared, the size of the gonad(s) should be noted by measuring, weighing or scoring. Assessing follicle development in the ovary also is important.

Other observations on the reproductive tract can provide additional information. A readily visible, welldeveloped, left oviduct usually indicates that the bird has laid eggs. For many species reliable data on oviduct size and appearance are lacking. The size of the organ should be recorded by measuring, weighing or scoring.

Study of the reproductive system can be supplemented by histological examination. The gonad and tract, or parts of them, should be fixed in buffered 10% formalin, and hematoxylin and eosin-stained sections should be prepared. After measuring and weighing, the reproductive organs can be fixed for study at a later date.

Weighing organs, especially the liver, heart, spleen, kidney and brain, is encouraged whenever possible. Changes in organ to body-mass ratios often occur during infectious and non-infectious diseases.

The retention of material following post-mortem examination, referred to frequently above, is important for several reasons:

■ It may be necessary to go back to the carcass later in order to carry out additional investigations. This may prove necessary, for example, if histopathology suggests a bacterial infection, in which case unfixed samples can be taken and cultured to identify the causal organisms.

• Carcasses or other material may be required for legal (forensic) purposes, if, for example, a court action relating to the bird's death is to be brought (Cooper and Cooper 2007).

• Material may be needed for research. This requirement can range from whole bodies, study skins, or skeletons for museums, to the retention of relevant samples for morphometric study of gross or microscopic anatomy. In some cases, the bird's carcass and or tissues may be needed for a reference collection (see below).

The likely fate of carcasses, tissues and specimens should be assessed initially before the examination is conducted. Appropriate containers will be needed, and a decision must be made as to how to dissect the bird and preserve its body and tissues. For example, tissues for histology can be stored in 10% buffered formalin, but this method will destroy most microorganisms and damage DNA. Freezing, on the other hand, will preserve most microorganisms and DNA but will hamper histological and electron microscope work. Plastic and glass containers may influence results if they are used to store samples for certain toxicological analyses.

Facilities for storing carcasses and tissues may be limited, in which case a decision has to be made as to what is retained and for how long. As a general rule, following a post-mortem examination, the bird's carcass and tissues can be kept in a refrigerator at 4°C for up to 5 days, after which, if still needed, they should be frozen at -20°C, or fixed in formalin, ethanol, or a combination of both. Material from threatened, endangered, or endemic species should be retained for future reference or retrospective studies (Cooper and Jones 1986, Cooper et al. 1998). If a specific reference collection for the species exists, the carcass, except for small portions of tissue, including the liver, should be fixed in formalin. The latter should be frozen or fixed in ethanol.

Considerations for necropsying neonates and eggs. The examination of young, neonatal raptors is not as straightforward as it may seem. They are not simply smaller versions of the adult bird. The nestling's immune system is just beginning to develop and respond to antigens in the environment (see below). Its powers of thermoregulation usually are poorly developed, especially in nidicolous species such as raptors. These and other features mean that susceptibility to certain infectious agents, as well as to physical factors such as cold, may be enhanced. Investigation of the young bird should follow standard techniques for "neonates" that were originally developed for domestic poultry (Cooper 2002). An important feature of the necropsy of young birds is the examination, measurement, and sampling of the bursa of Fabricius. This organ, which lies adjacent to the cloaca, is a key component of the immune system and its investigation is imperative if mortality and morbidity of young birds is to be fully investigated. The bursa as well as the thymus, another part of the immune system, should be examined, weighed or measured and fixed in formalin for subsequent examination. If an investigator is in doubt over the examination of young birds, they should seek the advice of an experienced avian pathologist. This also applies to necropsying eggs and embryos (see below).

The comprehensive examination of raptor eggs is highly specialized. Most information in this area comes from studies involving domestic fowl and other galliforms and, more recently, passerines and psittacines (Cooper 2002, 2003a). Unfortunately, the examination of eggs often does not follow a standard protocol. Toxicologists, for example, examine and take samples differently from pathologists, who are particularly interested in infectious diseases, developmental abnormalities, and incubation failures. A detailed description of specific techniques for examining eggs appears in Appendix 3, and a recommended report form is provided in Appendix 4. Measuring eggshell thickness is an important part of assessing eggs, whether or not the eggs are fertile. Various methods can be used. A useful index is described in Ratcliffe (1970). Eggshells should be stored dry for future reference.

Laboratory Investigations

Laboratory investigation of samples is an important component of clinical work, as well as an essential component of necropsy examination and a useful adjunct to environmental studies. An extensive range of tests is available depending upon the situation and resources available. For example, carcasses of raptors found near a chemical spill are likely to undergo toxicological analyses rather than cultured for bacteria, fungi, or viruses. Unfortunately, laboratory procedures are expensive and the cost of some may be prohibitive. Funding may permit only a limited number of tests on a sample of birds, with the remainder being stored for investigation later. When this occurs, researchers should store the carcasses and tissues appropriately (see above). This includes safety concerns. Glutaraldehyde, for example, which must be stored below 40°C if it is not to deteriorate, is toxic to humans and must be handled accordingly. Examples of investigative tests on whole birds (both live and dead) and tissues are given in Tables 2 and 3, respectively. Although a few of the techniques listed can be learned quickly (e.g., detecting of helminth and protozoan parasites, preparing cytological preparations, etc.), others will require technical assistance.

Table 2. Investigative tests on live and dead birds.

Investigative test	Live birds	Dead birds
Clinical examination	+	-
Post-mortem examination	-	+
Radiology	+	+
Hematology	+	+/- ^a
Clinical chemistry	+	+/-
Microbiology	+	+
Toxicology	+/-	+
Histology	+/-	+
Electron microscopy	+/-	+
Chemical analysis of carcass	-	+
-		

a of limited value

Tal	ble	3.	Laboratory	tests o	n samples	from	raptors.
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Samples	Available from	Comments	
Blood in appropriate anticoagu- lant for hematological and clini- cal chemical analysis and detec- tion of hemoparasites.	Usually only from live birds; occasionally, small samples can be retrieved from birds that died very recently.	Various blood tests can be conducted, and databases of reference values are being established. The subject is a specialized one and reference should be made to standard texts including Campbell (1995) and Hawkey and Dennett (1989). Blood smears also can be valuable, but experience is needed to produce good preparations and the possibility of error, especially when looking for and quantifying hemoparasites, is high. Consult Cooper and Anwar (2001), Feyndich et al. (1995), and Godfrey et al. (1987).	
Blood without anti-coagulant (serum) for serological investi- gation.	Usually only from live rap- tors; occasionally small samples can be retrieved from birds that have died very recently.	Serology, usually to detect antibodies to viruses and other organisms, has an important part to play in both disease diagnosis and health monitoring. Various serological tests are available and each demands skill in performance and interpretation. A rise in antibody titer usually is considered indicative of exposure to a specific organism. The increase, however, can take time and may not be apparent in birds that have only recently contracted an infection.	
Tissues fixed in 10% formalin (preferably buffered) for histology.	Dead birds; occasionally live biopsies, but usually only from a dermal lesion or one that is surgically	Fixed tissues can be stored indefinitely and examined at a later stage. The general rule is to take lung, liver, and kidney (LLK) tissue, plus any organs that show abnormalities or which are considered important because they may provide useful information (e.g., bursa of Fabricius and thymus of young birds, which can yield data on immune status).	
	accessible.	Samples, usually, should not exceed 20 millimeters ² and fixative volume should be ten times that of the tissue.	
		Small carcasses can be fixed whole, following opening for processing.	
Tissues fixed in glutaraldehyde for transmission electronmi- croscopy (TEM).	As above.	Generally as above, but only tiny samples are taken. Scanning electronmicroscopy (SEM) employs different techniques and is not considered here.	
Cytological preparations.	As above.	Easy to take and inexpensive to process (readily done in any veterinary practice or in the field). Produces results rapidly. Usually consist of touch preparations or impression smears which can give valuable information about tissues within a few minutes. The samples first must be blotted on filter paper to remove excess blood.	
Swabs, organ and tissue sam- ples, and other specimens for microbiological and other investigations.	Live or dead birds, dermal lesions, mouth or cloacal swabs, internal organs (car- casses only).	Usually sampled with swabs (in transport medium if they are to be sent elsewhere). Includes por- tions of tissue as well as exudates and transudates (Hunter 1989, Scullion 1989). If culture is not possible, an impression smear stained with Gram or other stains often provides useful information.	
Tissues for toxicological exami- nation.	Dead birds mainly, but some small samples can be taken from live birds as well (e.g., blood or muscle biopsies for pesticide analysis, and feathers for heavy metal and other analyses).	It is important that samples from wild bird casualties are taken and stored routinely for toxicologi- cal analysis. Samples for toxicology usually are kept frozen for later analysis. Samples should be taken and stored even when there is no immediate prospect of their being analyzed.	
Droppings, including feces and urates as voided, for parasito- logical and other tests.	Both live (recently voided droppings) and dead birds (removed from the cloaca).	Droppings provide a means of diagnosing some diseases and obtaining health-monitoring data with minimal disturbance to the live bird (Cooper 1998). Droppings often are passed when a raptor is restrained or handled. The fecal component can be used to detect internal parasites, to provide information on other changes in the intestine (e.g., the presence of blood, undigested food, etc.) or to investigate the origin of recently ingested food. Feces also can be used to detect the antigens of pathogenic organisms and to provide other information based on DNA technology. The urate component of feces can be used to investigate kidney function and also may yield parasites associated with the renal system. In all cases, fresh samples provide the most reliable results.	
Stomach and crop contents.	Usually from dead birds. Stomach and crop washings can be obtained from live birds or regurgitation can be stimulated by physical or chemical means. Regur- gitated pellets can provide valuable information.	As above.	
Feathers.	Both live and dead birds.	Can be examined for lesions, analyzed for heavy metals, and used in studies involving mitochon- drial DNA (Cooper 2002).	



Figure 2. Sample taking during post-mortem examination of birds.

One difficulty often faced is deciding which specimens to keep and how they should be preserved. Figure 2 illustrates the range of possibilities for some postmortem samples and the various methods used. When material is sparse, a "triage" system may need to be instituted.

Interpretation of Findings

The analysis and interpretation of results can present problems. For example, one cannot assume that a firearm killed a hawk that has lead shot in its body. The shot may be longstanding, related to a previous nonfatal, shooting and of no relevance to the bird's death. One also must distinguish between the cause of death and factors that may have contributed to it (i.e., the "proximate" versus "ultimate" causes). For example, a bird with avian tuberculosis or pox may become so weak that it is unable to hunt and as a result, is killed while scavenging by the roadside; the bird in question will have died of trauma, but the most significant pathological finding would be acid-fast *Mycobacterium* organisms in its internal organs.

Finding micro- or macroparasites on or in a bird also can be misleading. Sometimes parasites are acquired from prey species (e.g., lice from corvids), or from another carcass in the post-mortem room. Even when such organisms are bona fide isolates, their relevance may not be clear. Intestinal worms associated with hemorrhage in a bird's intestine, or bacteria isolated from a hot, swollen foot, clearly are likely to be of some significance, but what if such organisms are found without such lesions? Are they of importance? Much remains to be learned about the biology of pathogens (Reece 1989) and host-parasite relations (Cooper 2001) in free-living birds. Until that happens, it is best to record findings, both qualitatively and quantitatively, and to attempt to relate these to the bird's body condition and its systemic health. In this regard, data from captive raptors can provide useful references for wild bird casualties (Cooper 2003b).

The cause of death is "euthanasia" when the bird

has been killed on humanitarian grounds or to obtain fresh material for examination. In such cases, the aim of any investigation is to detect underlying lesions or factors that may have contributed to the bird's ill health or influenced its behavior.

Interpretation of pathological findings is particularly difficult. Mistakes can be made easily by those who are unfamiliar with the various disciplines involved. Thus, the profuse growth of a potentially pathogenic bacterium from a carcass does not necessarily mean that the organism was the cause of death; if the bird has been dead for some time it may have invaded the tissues post mortem. Likewise, the detection of a distinct pathological lesion, such as an interstitial nephritis, need not indicate that the raptor died of kidney disease; the renal damage may be chronic and not sufficiently severe to have proved fatal. In all cases, careful collation of results is necessary, and diagnosis and conclusions should be made only in the light of all information and findings available. Records are essential and, if possible, should be computerized to facilitate retrieval and analysis. Field and other preliminary data also should be retained. It is important to recall that in health studies on raptors a "diagnosis" is not necessarily the objective. Apparently minor background findings of parasitism or unusual gonads, for example, may be far more relevant, especially when the study is part of a larger, population-monitoring program.

From the above it is clear that care must be taken with regard to terminology. A "diagnosis" is one thing, the "cause of death" another, and underlying health-status yet another. Gross and laboratory findings need to be interpreted in the context of the background, history, and circumstances under which the birds were found and examined, the species and sex and age ratios involved, and other extraneous factors, including weather, that may have played a part.

Interpretation of findings also can be hampered by the lack of reliable reference values. For example, recently there have been great advances in our knowledge of the hematology and blood biochemistry of birds of prey, however the data available largely relate only to species that are kept or bred in captivity, or have been subjected to detailed study in the wild, and for some species little or no information is available (cf. Tryland 2006). Likewise, toxicological investigations can be thwarted because of a paucity of "normal" background values, as well as sub-lethal and lethal values for a given species. Although extrapolation is sometimes possible, it is far from ideal.

The absence of basic data remains a cause for concern. For instance, the normal ranges of organ mass and organ to body-mass ratios of most species of raptors are not known, and yet such information could be gathered easily if proper records were kept and findings freely disseminated. There is a need to involve scientists from all disciplines, including undergraduate and postgraduate students and "amateur" naturalists, in filling such gaps in our knowledge. Comprehensive databases on host-parasite relations of different families of birds also are needed (Cooper 2003b). These should encompass basic biological parameters of raptor hosts, as well as information about the macro- and microparasites associated with particular species, whether or not the latter are considered to be pathogenic. A useful first step is to compile local, national, and regional checklists of parasites together with the names of the hosts with which they are associated.

These caveats aside, several useful references for interpreting laboratory findings do exist. They include Randall and Reece (1996) on histopathology, Hawkey and Dennett (1989) and Campbell (1995) on hematology, and Scullion (1989) and Cooper (in Fudge 2000) on microbiology.

Legal Considerations

In the United Kingdom and several other countries, the making of a formal diagnosis, even as a result of examining a dead bird, is restricted by law to the veterinary profession (Cooper 1987). There are other legal considerations in raptor pathology as well. Health and safety legislation may dictate how and where clinical examination, sample taking or a post-mortem investigation is performed. Where a zoonotic disease is suspected, the legislation may demand a risk assessment and, perhaps, that the necropsy is only performed if appropriate protection (i.e., clothing, equipment, and facilities) is available for all those involved, and that the personnel are appropriately experienced or trained. Laws may restrict the movement of carcasses or specimens (Cooper 1987, 2000). Within countries, such laws usually relate primarily to postal requirements for adequate packing and transport. When moving samples from one country to another, the situation becomes more complex because conservation legislation, especially CITES (Convention on International Trade in Endangered Species), may apply. The Ministry or Department of Agriculture of the receiving country is likely to require documentation describing the type of material that is being transported,

particularly its likely pathogenicity. If the raptors in question are covered by CITES, there will be an additional need for permits. In addition, the movement of small specimens, including blood smears, or tissues for DNA study, remains a cause of frustration for those that wish to send samples to colleagues or laboratories in other countries. Even the smallest sample can fall into the category of a "recognized derivative" under CITES and, therefore, require appropriate documentation and authorization. Recently, there have been moves to obtain exemptions for such material, especially if the samples in question are required for important diagnostic or forensic purposes. CITES continues to debate the issue and, at the time of writing, the likely outcome appears to favor introducing a "fast-track" system for small, but urgent, samples (see Chapter 25). Those involved in health studies on birds of prey should be familiar with the relevant legislation and adhere to it.

In many countries, legislation relevant to health studies on raptors is non-existent or is poorly enforced. In such circumstances, it is good practice to work toward "in-house" protocols and to develop and use guidelines that, although not legally binding, help to ensure high standards of work (Cooper 1996). In all instances, the status of raptor biology is not served by breaching the law or broadly established professional protocols, however tedious and inconvenient they may appear.

CONCLUSIONS

Health studies are an important component of raptor management, both in the wild and in captivity. Of particular and increasing significance is health monitoring. Those working with raptors need to be aware of developments in this field, especially the new technology that is now available for the detection of organisms and antibodies.

The value of an interdisciplinary approach to the study of the diseases and health parameters of raptors cannot be over-emphasized. For centuries, in Europe, Arabia, and the Far East, it was the falconers, who kept and flew birds of prey, who knew most about the natural history of raptors and how to detect early signs of ill health in their charges. These people always maintained that keeping a hawk in good health was preferable to treating ailments, and many early texts advised on how this might be achieved through proper management (Cooper 2002). Charles d'Arcussia, the French nobleman, whose book on falconry was first printed in 1598 (Loft 2003), had a refreshingly positive approach to the question of disease and advocated the following: "If you want to maintain the health of your hawks take as guides those who are experienced and can lead you forward with their advice." This admonition remains relevant today. Raptor biologists have unprecedented access to literature, ranging from field notes and scientific papers to the Internet, and are able to take advantage of the numerous developments in clinical medicine and laboratory investigation that have characterized the past three decades. That said, we must remain wary of working in isolation and instead collaborate with others working in various disciplines that now contribute to our understanding of the health and diseases of birds of prey.

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Appendix 1.HEALTH MONITORING OF LIVE BIRDS OF PREY

Species:	Location:	Reference:	
Relevant history:			
Circumstances of monitoring			
Numbers of birds involved:		Details:	
Personnel involved:			
Other comments:			
OBSERVATION			
Behavior:			
Bird unaware of observer:			
Bird aware of observer:			
EXAMINATION			
Clinical signs:			
Injuries or external lesions and disting	guishing features:		
Plumage, molt, and preen gland:			
Ectoparasites:			
Species:			
Numbers:			
Body mass:		_ Carpal length:	
Other measurements:		Condition score:	
Samples			
Feathers:			
Feces:			
Swabs:			
Blood:			
Others:			
Follow-up tests			
Reported by:		Date:	Time:
Assisted by:			

Appendix 2. POST-MORTEM EXAMINATION (NECROPSY) OF DEAD BIRDS OF PREY

Species:	Reference No:
Date of submission:	Origin:
Band (ring) number:	Other identification:

Relevant history and circumstances of death:

Request (category of necropsy): diagnosis (cause of death or ill-health), health monitoring, forensic investigation, research, or other:

Special requirements regarding techniques to be followed, instructions regarding fate of body or samples:

Submitted by:	Date:
Received by:	Date:

MEASUREMENTS	Carpus:	Tarsus:	Other:	Body mass:
Condition score: Ob	ese or fat / good / fair	or thin / poor		
State of preservation:	Good / fair / poor / r	marked autolysis		
Storage since death:	Refrigerator / ambien	t temperature / froze	n / fixed	

EXTERNAL OBSERVATIONS, including preen gland, state of moult, ectoparasites, skin condition, lesions, etc.:

MACROSCOPIC EVALUATION on opening the body, including position and appearance of organs, lesions, etc.:

ALIMENTARY SYSTEM: MUSCULOSKELETAL: CARDIOVASCULAR: RESPIRATORY: URINARY: REPRODUCTIVE: LYMPHOID (including bursa and thymus): NERVOUS:

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Appendix 2. continued. OTHER SAMPLES TAKEN Hist Cytology Bact Paras DNA Other (e.g., serology) Hist DNA Cytology Other (e.g., serology) Bact Paras _____ Hist Cytology Other (e.g., serology) Bact Paras DNA Cytology Other (e.g., serology) Bact Paras Hist DNA Hist Cytology Other (e.g., serology) Bact Paras DNA Hist DNA Cytology Other (e.g., serology) Bact Paras Other (e.g., serology) Paras Hist DNA Cytology Bact LABORATORY FINDINGS Date: _____ Initials: _____ Reported to whom: _____ PRELIMINARY REPORT (based on gross findings and immediate laboratory results, e.g., cytology) Reported to: Date: Time: FINAL REPORT (based on all available information) FATE OF BODY / TISSUES Destroyed / frozen / fixed in formalin (other) / retained for Reference Collection / sent elsewhere FATE OF RING/BAND (if appropriate) PM examination performed by: _____ Date: ____ Time: ____

Assisted by: _____

Appendix 3. PROTOCOL FOR EXAMINATION OF UNHATCHED EGGS OF BIRDS OF PREY



Appendix 4. EXAMINATION OF EGGS AND EMBRYOS OF BIRDS OF PREY

Reference number:		
Received (date):	(by):	
Receipt acknowledged by:	Da	te:
Method of packing/wrappings:		
History:		
EGG / EMBRYO EXAMINATION (to be co	ompleted for each specimen)	
Species:		
Owner / Origin:		
Weight of whole unopened egg:	Length:	Width:
External appearance:		
Appearance on candling:		
Embryo		
Air cell		
Blood vessels		
Fluids		
Appearance when opened:		
Contents:		
Embryo:		
Length (crown-rump)		
Amniotic cavity		
Allantoic cavity		
Yolk sac		
Other comments:		
Microbiology:		
Histopathology:		
Other tests:		
Samples sent elsewhere:		
Weight of dried eggshell:	Thickness (measurement or	index):
Samples stored:		
COMMENTS		
Examination performed by:	Date:	Time:
Assisted by:		