Pathology

17

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 well-being, 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 diag-

nosis. 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 much-debated role of various organisms in the biology of free-living 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.

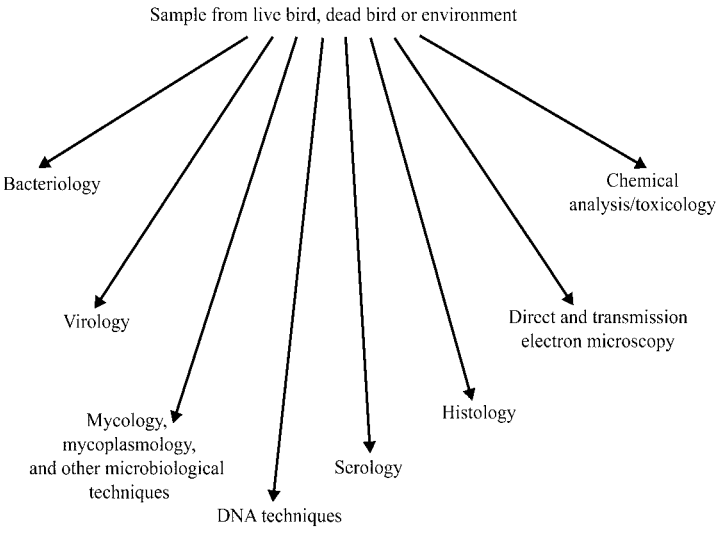

Figure 1. Sample from live bird, dead bird, or the environment.

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 post-mortem 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, Harcourt-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 post-mortem 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, well-developed, 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.

Table 3. Laboratory tests on samples from raptors.

Samples	Available from	Comments
Blood in appropriate anticoagulant for hematological and clinical chemical analysis and detection 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 investigation.	Usually only from live raptors; 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 accessible.	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). 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 electronmicroscopy (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 samples, and other specimens for microbiological and other investigations.	Live or dead birds, dermal lesions, mouth or cloacal swabs, internal organs (carcasses only).	Usually sampled with swabs (in transport medium if they are to be sent elsewhere). Includes portions 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 examination.	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 toxicological 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 parasitological 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 bacteria, fungi and viruses. Molecular techniques, including PCR, now are being 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. Regurgitated 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 mitochondrial DNA (Cooper 2002).

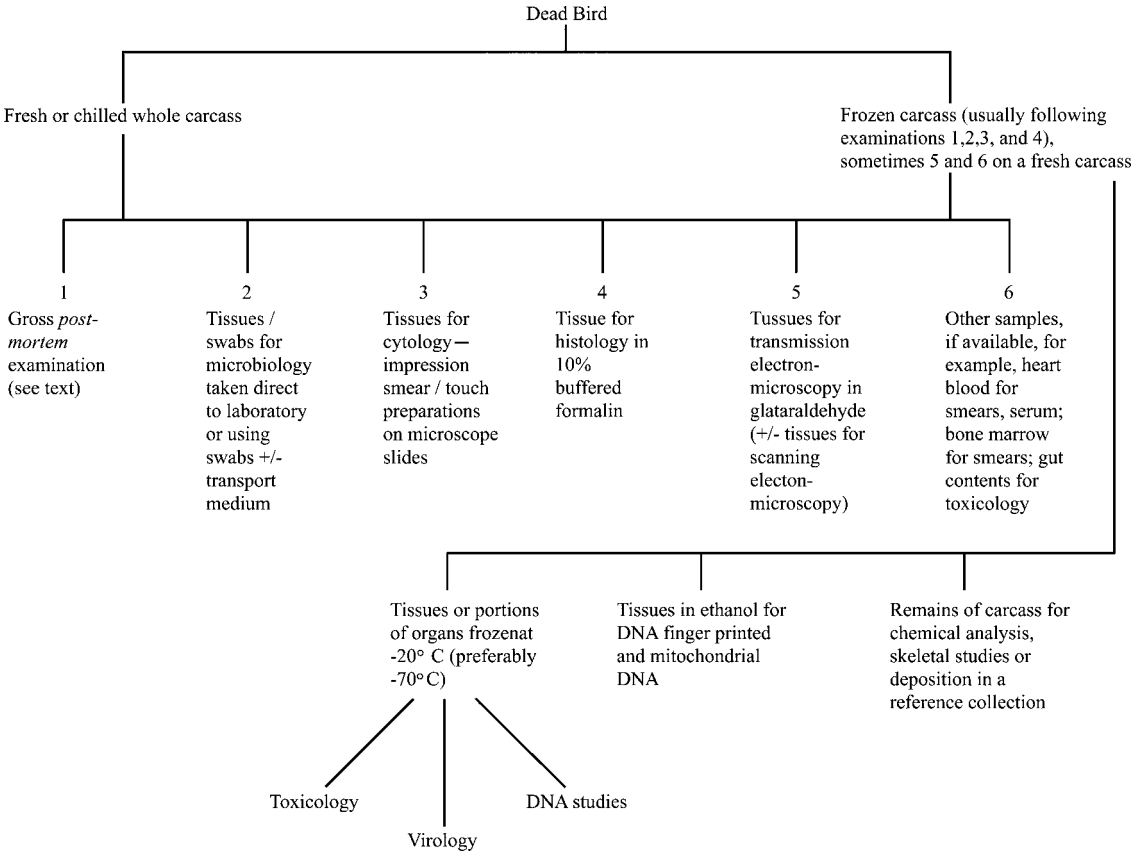

Figure 2. Sample taking during post-mortem examination of birds.

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 post-mortem 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 non-fatal, 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.

ACKNOWLEDGMENTS

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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 distinguishing 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: Obese or fat / good / fair or thin / poor

State of preservation: Good / fair / poor / marked autolysis

Storage since death: Refrigerator / ambient temperature / frozen / 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:

Appendix 2. *continued.*

OTHER SAMPLES TAKEN

_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)
_____	Bact	Paras	Hist	DNA	Cytology	Other (e.g., serology)

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

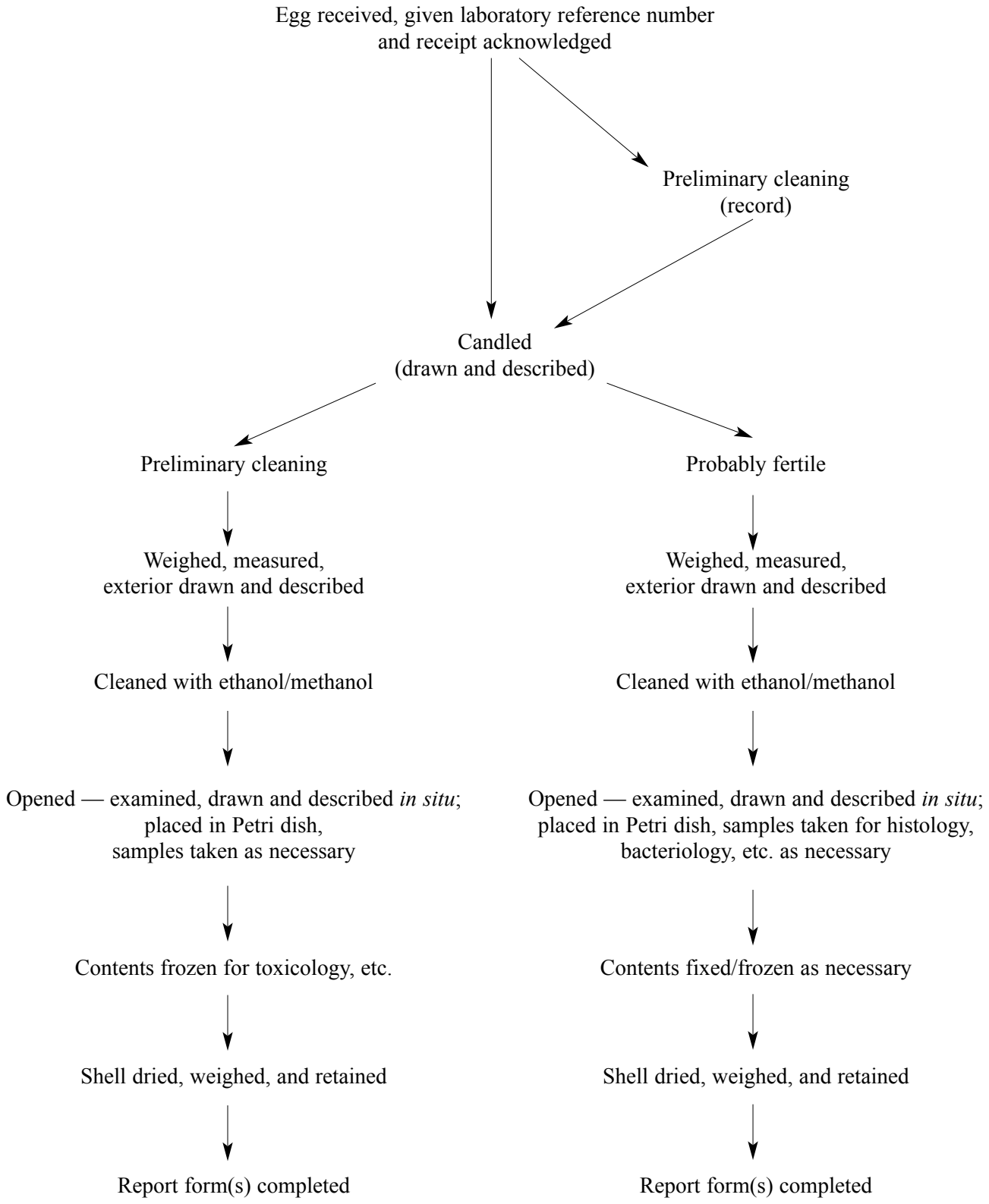
Destroyed / frozen / fixed in formalin (other) / retained for Reference Collection / sent elsewhere

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: _____ Date: _____

Method of packing/wrappings:

History:

EGG / EMBRYO EXAMINATION (to be completed 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:_____

B. Ectoparasites

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INTRODUCTION

Fly ectoparasites that feed on blood include biting midges, blackflies, blowflies, louse flies, mosquitoes, and carnid flies. Additional blood-feeding insects that parasitize raptors include cimicid bugs, fleas, and some chewing lice. Other chewing lice feed on feathers. Although usually nonparasitic, scavenging skin beetle (Dermestidae) larvae have even been found in wounds in Snail Kite (*Rostrhamus sociabilis*) nestlings in Florida, and other raptors in Africa and Europe (Snyder et al. 1984). Arachnid ectoparasites of raptors include blood-sucking ticks and mites, mites that feed on feather material, and mites, including chiggers, that feed on tissue. Most mites are external parasites, but some skin mites burrow into and under the skin, and some mites colonize the respiratory tract. In addition to direct pathological effects, raptor ectoparasites can have indirect pathological effects because a weakened host is more vulnerable to infection. Bacterial and fungal infections caused by ectoparasites can occur in wounds, and many flies, ticks, and mites act as disease vectors as well. Philips (2000, 2006a,b) reviewed the parasitic mites of raptors and the author maintains an online checklist of raptor hosts and their mite ectoparasites.

Levels of ectoparasite infestation vary greatly among and within species of raptors. Raptor ectoparasite management involves collection, preservation, and identification of ectoparasites, followed, when necessary, by treatment of affected birds and control measures to reduce the ectoparasite levels in the nest or local environment. Clayton and Walther (1997) reviewed collection and preservation techniques of avian ectoparasites. Beynon et al. (1996) list ingredient formulas for six ectoparasiticides useful in the treatment and control of insects and mites that parasitize raptors.

Raptors and their nests should be surveyed and monitored for ectoparasites as causes of direct pathology and disease transmission. Raptor ectoparasites, such as the lice of the threatened Galapagos Hawk (*Buteo galapagoensis*), can serve as excellent markers of host population differentiation (Whiteman and Parker 2005). Host-specific ectoparasites of endangered raptor species are themselves endangered species.

Insects and blood-filled ticks and mites are much more noticeable to the naked eye than most mites. Feather mites often look like grains of sand, and 0.25-mm chiggers and skin mites as "specks." Species identification often requires ectoparasite dissection, particular ectoparasite clearing techniques, particular slide-mount media, and specialized taxonomic expertise. The mite fauna of raptors is largely unknown, and many new species remain to be discovered. Below I detail the types of flies, cimicid bugs, fleas, chewing lice, ticks, and mites that parasitize birds of prey.

FLIES (DIPTERA)

Biting Midges (Ceratopogonidae)

Boorman (1993) provides an identification key to adults in blood-sucking ceratopogonid genera.

Biting midges, which often are called "no-see-

ums," transmit filarial nematodes, blood protozoans *Haemoproteus* and *Leucocytozoon*, and the Thimiri arbovirus to birds (Mullen 2002). After a blood meal, female midges lay eggs in habitats ranging from moist compost and manure to water in tree holes, freshwater marshes, and mangrove swamps. Females can be aspirated from hosts or collected by using light-traps with black-light lamps and carbon dioxide. Midges can be preserved in 1–2% formalin or 70–80% alcohol. Controlling these ectoparasites is difficult. Most screens are not effective and neither is general application of insecticides to kill larvae. Eliminating breeding habitat and general application of insecticides as mists or fogs in early evening when adults are most active can reduce populations.

Blackflies (Simuliidae)

Crosskey and Howard (1997) provide an inventory of the blackflies of the world. Blackflies are the main vectors of *Leucocytozoon* in birds, and also they transmit *Trypanosoma* and filarial nematodes (Adler and McCreadie 2002). Adler et al. (2004) list North American blackfly species, their raptor and other hosts, and the species of Leucocytozoon they transmit.

Blackflies have killed nestling Red-tailed Hawks (*B. jamaicensis*) (Brown and Amadon 1968, Smith et al. 1998), nestling Merlins (*Falco columbarius*) (Trimble 1975), and have weakened nestling Cape Vultures (*Gyps coprotheres*) (Boshoff and Currie 1981). Blackflies tend to feed on the crown, back, and shoulders of raptors. Biting occurs during the day in the open, and adult blackflies can be collected from hosts with an aspirator, or with sticky silhouette or carbon dioxide traps. After a blood meal, females lay their eggs in running water. Fluid preservation destroys important taxonomic features, so adults should be micropinned through the thorax after they dry in a freezer for 5 weeks (Crosskey 1993).

Blackfly control, which mainly targets the larvae, uses the entomopathogenic bacterium, *Bacillus thuringiensis var. israelensis*, applied to bodies of water by hand or from the air. Providing shelters for captive birds helps protect them from blackflies.

Mosquitoes (Culicidae)

The mosquitoes of the world are listed in Knight and Stone (1977) and its supplements (Knight 1978, Ward 1984, Gaffigan and Ward 1985, Ward 1992). There are many regional identification keys, including that of Darsie and Ward (2005) to species of the U.S. and Canada, and a key to world genera by Mattingly (1971).

Mosquitoes transmit many viruses to birds, including encephalomyelitis viruses, West Nile virus, and poxvirus (Foster and Walker 2002). They also are vectors of avian malaria (*Plasmodium*) and filarial nematodes. After a blood meal, female mosquitoes lay eggs on water or wet surfaces under floating vegetation or in the walls of wet tree-holes (Service 1993). Mosquitoes can be collected from hosts and in shaded resting places using aspirators, and with carbon dioxide traps and light-traps. Specimens should not be preserved in liquids but micropinned through the thorax.

Approaches to control of mosquito populations include reducing their breeding habitat; using light mineral oils, organophosphates, insect-growth regulators, or *Bacillus thuringiensis var. israelensis* to kill the aquatic larvae; applying residual insecticides to adult resting surfaces; and direct contact spraying or fogging of organophosphates, carbamates, pyrethrins and synthetic pyrethroids. Screens can protect captive birds.

Louse Flies (Hippoboscidae)

Maa (1963) lists the louse flies of the world and provides genera and species-group identification keys.

Avian louse flies, often called flat flies, tend to remain on their host unless disturbed, and they sometimes bite humans that handle infested birds. Larvae develop in the female and pupate in birds' nests and roosts immediately when born. Louse flies transmit the blood protozoans, *Haemoproteus* and *Trypanosoma*, through biting, and carry lice and the ectoparasitic skin mites, *Strelkoviacarus*, *Microlichus,* and *Myialges*, on their exterior to new bird hosts (Philips 1990, Lloyd 2002). Louse flies have tested positive for West Nile virus, but their role as vectors of this and other viruses is unconfirmed. Infestation of several dozen louse flies does not seem to harm raptors, but when levels exceed 80, raptors become emaciated and too weak to hunt. Louse flies, which range in size from 4 to 7 mm, can be caught with air nets and by hand, and can be pinned or preserved in ethanol. Infested birds can be treated with pyrethroid dust.

Myiasis Flies (Calliphoridae, Muscidae)

Sabrosky et al. (1989) provides a key and a host list for Nearctic *Protocalliphora*, and lists the Palearctic

species. Whitworth (2003, 2006) provides a species key to *Protocalliphora* pupae. Furman and Catts (1982) designed a key to a variety of myiasis-causing fly genera.

Nest flies of raptors include the Holarctic and Oriental blow flies *Protocalliphora* (Calliphoridae), the European carrion flies *Lucilia sericata* and *Calliphora* (Calliphoridae), and the tropical flies *Philornis* and *Passeromyia* (Muscidae), all of which lay their eggs in nests or on nestlings. The maggots of these flies cause myiasis by burrowing into host tissues and sucking blood (Baumgartner 1988). Ear cavities, noses, the ventral surface, and feather sheaths are preferred sites. After feeding, larvae drop off the host to digest their blood meal and pupate.

Myiasis is known to kill nestling Northern Harriers (*Circus cyaneus*) (Hamerstrom and Hamerstrom 1954), Sharp-shinned Hawks (*Accipiter striatus*) (Delannoy and Cruz 1991), Verreaux's Eagles (*Aquila verreauxii*) (Gargett 1977), Gyrfalcons (*F. rusticolus*) (Poole and Bromley 1988), and Prairie Falcons (*F. mexicanus*) (White 1963), and to weaken nestling Red-tailed Hawks (Tirrell 1978) and prolong their development (Catts and Mullen 2002). Burrowed larvae will evacuate nestlings if the breathing opening of the larvae is blocked with petroleum jelly or if nestling orifices are flushed with saline solution. Mineral oil can be used to remove them from ear cavities. Maggots should be relaxed before being preserved in ethanol (Hall and Smith 1993). This can be accomplished by placing them into water just below the boiling point, or into acetic alcohol (one part glacial acetic acid to three parts 90% ethanol). Dissecting nest material can yield pupae. Treatment involves removing larvae and applying antibiotics to the wound to prevent infection. Nests can be dusted with pyrethroids.

Carnid Flies (Carnidae)

Carnid flies can be identified using the fly family key of Arnett (2000). Grimaldi (1997) discusses the species, of which the most well known is *Carnus hemapterus*, and lists all avian hosts.

Carnus larvae scavenge in nests. Wingless adults either suck the blood of nestlings or feed on their skin secretions. Infestations are characterized by scabby axillae. Heavy infestations cause reduced pack-cell volumes in Barn Owls (*Tyto alba*) (Schulz 1986), reduced body mass in Common Kestrels (*F. tinnunculus*) (Heddergott 2003), and nestling mortality in Northern Sawwhet Owls (*Aegolius acadicus*) (Cannings 1986). The fly seems harmless to American Kestrels (*F. sparverius*) (Dawson and Bortolotti 1997). *Carnus* occurs in North America, Europe, Africa, and Malaysia. Specimens can be collected from hosts by hand or from nests by Tullgren funnel extraction of nest material (Mullen and O'Connor 2002), and then preserved in ethanol. Insecticide dusts can be used to treat hosts and control infestations in nests.

CIMICID BUGS (BED BUGS)

Cimicid bugs (Cimicidae) lay eggs where hosts live. Both adults and nymphal stages suck blood. One species in particular regularly attacks raptors. The Mexican chicken bug (*Haematosiphon inodorus*) has killed nestling Bald Eagles (*Haliaeetus leucocephalus*) (Grubb et al. 1986) as well as nestling Red-tailed Hawks and Prairie Falcons (Platt 1975, McFadzen and Marzluff 1996), and has caused nestling California Condors (*Gymnogyps californianus*) to fledge prematurely (Brown and Amadon 1968). The swallow bedbug (*Oeciacus vicarious*) occurs in Prairie Falcon aeries. The bugs hide in nests or cracks near hosts during the day, and feed mainly at night near the eyes and at the base of the host's legs and wings. Cimicid bugs can be collected with forceps, Tullgren funnel extraction, or dissection of nest material, or be forced out of cracks with pyrethroid or kerosene sprays (Schofield and Dolling 1993). Specimens can be preserved in ethanol. Usinger (1966) provides species identification keys for the family and an avian host list. Treatment and control involve spraying hosts, nests, and surfaces near the host with insecticides including pyrethrins.

FLEAS (SIPHONAPTERA)

Regional identification keys with host lists include Holland (1985) for Canada, and Benton and Shatrau (1965) and Lewis et al. (1988) for parts of the U.S. Lewis (1993) provides a key to medically important flea genera globally. Arnett (2000) provides an identification key to families, and Lewis (1993) provides more detailed keys to some of the taxa.

Fleas of adult raptors bite hosts to obtain blood and lay their eggs on their hosts or in nests, where larvae are scavengers. Typically, more fleas are found in nests than on hosts. One exception is the sticktight flea (*Echidno-*

phaga gallinaacea), which remains attached to hosts in unfeathered places around the head. Burrowing Owls (*Athene cunicularia*) in particular seem to be infested with fleas when nesting (Smith and Belthoff 2001). Fleas can be collected from hosts with insecticide dusts, and by dissecting nest material or via extraction in a Tullgren funnel. They can be preserved in 80% ethanol. Treatment and control involve pyrethrin dusts and insect growth regulators (Lewis 1993, Durden and Traub 2002).

CHEWING LICE (MALLOPHAGA)

Price et al. (2003) provide a list of avian lice globally, their hosts, and identification keys to genera by host.

Chewing lice usually are transferred by direct contact and, less frequently, by louse flies. Their feeding can damage feathers, and scratching in response to infestation can cause additional damage. Heavy louse infestations cause anemia, weight loss, and death. Lice can be collected from hosts with forceps, or by ruffling feathers after dusting with insecticidal powder (Clayton and Drown 2001). During necropsy, carcasses can be washed with detergent or skinned, and skin and feathers dissolved using trypsin or potassium hydroxide (Furman and Catts 1982). Detergent washes also will yield mites, whereas dissolving tends to destroy most mites. Resulting solutions are sieved or filtered to collect specimens. Specimens should be preserved in 95% ethanol. Insecticidal dusts and resin strips are useful in treatment and control (Durden 2002).

TICKS (IXODIDA)

Varma (1993) provides an identification key to tick families and genera.

Larval, nymphal and adult ticks all suck blood, often from different hosts. Individuals remain attached to hosts for as long as two days (Sonenshine et al. 2002). Eyelids and the bases of beaks are usual feeding sites. Most ticks are ambush parasites found in litter and soil that latch on to passing hosts. Avian soft ticks (Argasidae — *Argas* and *Ornithodoros*) and some hard ticks (Ixodidae — *Ixodes*) live in nests and burrows. Ticks transmit avian spirochetosis and Lyme disease, and are vectors for *Babesia* spp., an anemia-causing protozoan known to occur in Prairie Falcons (Croft and Kingston 1975). They also transmit viruses and tularemia bacteria to birds. Some species produce a toxin in their saliva that induces paralysis. Ticks have killed nestling Prairie Falcons (Webster 1944, Oliphant et al. 1976) and Peregrine Falcons (*F. peregrinus*) (Schilling et al. 1981), and tick paralysis killed an adult Powerful Boobook (*Ninox strenua*) (Fleay 1968) in Australia. Ticks can be collected directly from hosts by dissecting nest material by extraction with a Tullgren funnel, by dragging a blanket or sheet over vegetation, and with carbon-dioxide traps. Ethanol preserves soft ticks, and Pampel's fluid (2 ml glacial acetic acid, 6 ml 40% formalin, 30 ml distilled water, and 15 ml 95% ethanol) prevents hard tick scutal patterns from fading.

Ticks should be removed carefully from hosts with forceps, making certain to avoid leaving the mouthparts embedded in the skin. A drop of ethanol or oil can be used to detach individuals. Antibiotics should be applied to the point of attachment once the tick has been removed. Pyrethroid dusts are useful in control.

MITES (ACARINA)

Blood-sucking Mites

Varma (1993) provides an identification key to the most important species of *Dermanyssus* and *Ornithonyssus*.

Nidicolous mites in the genera *Dermanyssus* and *Ornithonyssus* and their less common relatives, as well as rhinonyssid nasal-cavity mites, feed on blood. Rhinonyssid nasal-cavity mites that cause rhinitis or sinusitis usually are limited to a few individuals per host (Mullen and O'Connor 2002). *Sternostoma* can clog air sacs, causing wheezing and mortality. *Dermanyssus* and *Ornithonyssus* populations can mass on hosts, causing anemia and weight loss. Tropical fowl mites (*Ornithonyssus bursa*), which usually feed near the vent, have killed nestling Snail Kites (Sykes and Forrester 1983) and a captive adult Eurasian Sparrowhawk (*A. nisus*). *Ornithonyssus* transmits encephalitis viruses, and *Dermanyssus* transmits the white blood cell-infecting protozoan *Lankesterella* (Box 1971). Nasal mites can be collected from live hosts by flushing the nares with water, whereas *Dermanyssus* and *Ornithonyssus* can be obtained by ruffling feathers dusted with insecticide powder, or from nest material by dissection or extraction using a Tullgren funnel. Mites should be preserved in Oudemans' fluid (5 parts glycerine, 8 parts glacial acetic acid, and 87 parts 70% alcohol) to prevent hardening. Treatment and control of external mites involves

pyrethroid and other insecticide dusts or sprays. Rhononyssid mites can be controlled with dichlorvos pest strips or pyrethrin-piperonyl butoxide spray (Ritchie et al. 1994).

Skin and Tissue-eating Mites

Skin-mite identification keys are outdated, incomplete, scattered or in some cases, nonexistent. Krantz (1978) provides family keys for mites, overall.

Skin or tissue-eating mites on raptors include *Pneumophagus* in the lungs and air sacs, Ereynetidae in the nasal cavity, Turbinoptidae in the outer nares, Hypoderatidae under thigh and underbody skin, Syringophilidae in quills, and Analgidae, Cheyletiellidae, Epidermoptidae, Harpirhynchidae, Knemidocoptidae, and Trombiculidae (chiggers) on or in the skin. Cheyletiellid mites also feed on blood, and, as with epidermoptid and harpirhynchid mites, can cause edema, hyperkeratosis, and feather loss, with secondary infections in skin lesions. *Knemidocoptes* can cause development of scaly-face and scaly-leg encrustations. Females of *Strelkoviacarus* and *Microlichus* are phoretic on louse flies, whereas *Myialges* females lay their eggs on these flies. Hypoderatid mites reproduce in nests, but their adults are nonfeeding and short-lived. Chiggers, often a cause of dermatitis, are larval mites whose nymphal and adult forms are soil predators. Skin mites can be collected from hosts in skin scrapings, and with detergent washes during necropsies. Hypoderatid mites may be revealed as lumps under the skin. Chiggers can be collected by placing a black disk on the ground below the bird, which will attract them (Mullen and O'Connor 2002). Skin and tissue-entry mites can be preserved in Oudemans' fluid. Ivermectin can be used to treat infestation of nasal, skin and syringophilid quill mites.

Feather-eating Mites

Thirteen families and 22 genera of feather-eating mites parasitize raptors. Gaud and Atyeo (1996) provide keys to genera of the feather mites of the world.

Many mites live on feathers where they scavenge fungi, lipids, bacteria, and feather fragments. A few live in the rachis and quill and eat medulla tissue. Feather and quill mites are most abundant on wing feathers. Feather mites can be collected by ruffling feathers dusted with insecticides. Most quill mites require dissection of shed feathers or quills during necropsy. Oudemans' fluid can be used for preservation. Pyrethrin dusts reduce feather mite populations, while dichlorvos pest strips or ivermectin can be used to treat quill mite infestations (Ritchie et al. 1994).

Feather Microbiology

The microbiology of raptor feathers is poorly known. Hubalek (1974a,b, 1981) surveyed the keratinophilic and other fungi on Common Kestrels and European owls, whereas Rees (1967) found two fungal genera on the feathers of Australian raptors. Pinowski and Pinowska (unpubl. data) have reviewed the feather fungal literature, and concluded that feather fungi are not very important in that they remain mostly dormant and rarely destroy feathers, and do not regulate the numbers of other feather ectoparasites. Bacteria also degrade feathers (Goldstein et al. 2004), but Cristol et al. (2005) found no evidence that they affect feathers on living birds. Although many North American birds have been examined for these bacteria (Burtt and Ichida 1999, Muza et al. 2000), raptors have yet to be studied in this regard.

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C. Endoparasites

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INTRODUCTION

Endoparasites are organisms that, in their developmental or adult stages, live in animals called hosts. Endoparasites, which include single-celled protozoa, worms (helminths), and arthropods, invade nearly all organs of animals. Protozoans are found in digestive and respiratory systems, muscles, blood, and feces of their hosts. Several endoparasitic worms feed on the ingesta in the intestine of the definitive (final) host or are attached to the mucosal layer within the intestine or the trachea where they suck blood or epithelial cells. Other worms are found in specific organs or only parts of organs. Some worms migrate through different internal organs during their stages of development. Parasitic arthropods, including ticks, mites, flies, mallophages, and fleas, often are found on the skin or feathers of their hosts. Only a few arthropods enter the internal organs of the hosts. Endoparasitic arthropods include mites living in layers of the skin or subcutaneously, and the larval stages of flies (maggots) that burrow through internal organs.

It is not the aim of this chapter to describe all endoparasites and their ways of life but rather to provide information on several relevant examples.

The traditional doctrine in parasitology states that a good parasite does not harm its host in a way that it weakens or kills the host, because this also would affect the parasite itself. And indeed, long-term, well-adapted parasites often are less pathogenic to their traditional hosts, whereas evolutionarily young parasites can harm their hosts severely. That said, the host–parasite relationship is a dynamic evolutionary system that may be compared to an arms race, in which both sides alter their behavior in response to the other in a way that sustains the interaction (Van Valen 1973). Dobson et al. (1992) described parasitic worms as natural enemies causing a permanent drain of energy in their hosts that affects the behavior and reproductive success of them. Depending on age, immune status, and infection pressure, parasites can invade their hosts to different degrees, and probably are a strong selective force on their hosts.

Parasitism as a way of life developed independently in different taxa. Endoparasites are believed to have evolved several million years ago. The oldest parasitic roundworms (nematodes) were found in beetles embedded in amber from the Eocene (Conway Morris 1981). Compared with their hosts, parasites are relatively simple organisms, many of which have "degenerated" during evolution, although parasites have the advantage that processes such as digestion or locomotion are provided by the host. Tapeworms living in a nutritionally rich environment — the intestine of their hosts — have reduced their digestive system and are able to reabsorb their food through their cuticula. Parasites also have developed new abilities in response to their parasitic lifestyles (e.g., host-finding mechanisms, resistance against the host's immune and digestive system, and new organs such as sensoric receptors). As a result of

such adaptations, the genome of parasites can be bigger than those of the free-living parasite relatives and, in some cases, bigger than those of the hosts they occupy (Poulin 1998).

As an adaptation to their way of life endoparasites often develop complex life cycles, including stages of sexual and asexual reproduction. Sexual multiplication normally occurs in the definitive host they forage in. The developing stages of endoparasites leave the host actively or passively to move to the next suitable environment. The life cycle of a parasite can be direct or indirect (i.e., via intermediate hosts). Complex life cycles often contain more than one intermediate host required by the parasite to reach the definitive host. Sexual reproduction generally occurs in the definitive host and asexual reproduction occurs in intermediate hosts. On the way to the next host many developmental stages may be lost. To increase the likelihood of host infection, parasite fecundity often has increased during its evolution. As a result, some roundworms can produce about 200,000, and some tapeworms up to 720,000 eggs per day (Crompton and Joyner 1980).

Parasites have developed a diversity of strategies to reach their definitive host. They often try to produce as many eggs as possible of which only a few develop to mature parasites. Sometimes intermediate hosts are manipulated by their parasites to become an easier victim to a predator. Several species of flukes that parasitize piscivorous birds migrate into the eyes of the last intermediate host (fish) reducing their ability to see (Odening 1969). Protozoa of the genus *Sarcocystis* use raptors as definitive hosts and mice or birds as intermediate hosts where they form cysts in the muscles. Infected mice changed their behavior and are twice as likely to be eaten by Common Kestrels (*Falco tinnunculus*) as are non-infected mice (Hoogenboom and Dijkstra 1987). Behavioral changes due to parasitic infections in definitive hosts (birds of prey) also have been described. Experimentally infected American Kestrels (*F. sparverius*) exhibited decreased flight activity during their reproduction and a prolonged courtship behavior compared to control birds (Saumier et al. 1991).

Whether an infection with parasites induces clinical signs depends on the status of the immune system, hormone status, and infection pressure. An infection with a few ascarids, for example, may cause some irritation in the intestinal mucosa but may not necessarily affect the condition of the host. A heavy infection can block the lumen partially or completely, resulting in a perforation or rupture. The metabolic products of worms also can harm the health of the host. Continued parasitic infection can weaken the host's immune system, thereby enabling additional parasites to enter the host. In such instances the parasitic infection is considered a factorial disease.

ENDOPARASITES OF BIRDS OF PREY

Endoparasites are frequently detected in raptors. Indeed, in some populations of birds of prey, 90% of all individuals have helminths (e.g., Krone 2000, Sanmartin et al. 2004).

Endoparasites found in birds of prey include protozoans, roundworms (nematodes), spiny-headed worms (acanthocephala), flukes (digenetic trematodes), tapeworms (cestodes), and tongue worms (pentastomida).

I provide a broad introduction here. A more extensive review of endoparasites found in birds of prey is in Lacina and Bird (2000). A more general overview of helminths in birds is in Rausch (1983). For information on the biology and treatment of parasites in raptors see Krone and Cooper (2002).

Protozoa

Unicellular parasites usually are very small and only visible by a microscope (Fig. 1). Among the eight classes of protozoa, two are of major interest in raptor parasitology.

Trichomonas. One of the oldest recognized and most significant diseases in raptors, "frounce" or "crop canker," is caused by *Trichomonas gallinae*, a single-celled organism that belongs to the class Zoomastigophorea. These spindle- to pear-shaped small flagellated protozoans reproduce by simple division. Direct transmission via the feeding of nestlings with crop-milk occurs in Columbiformes. The parasite lives on and in the mucosal layers of the oropharynx, oesophagus, and crop. Infection in raptors occurs via the ingestion of contaminated prey. Pigeons and doves are the main reservoirs of *Trichomonas*, but other birds, including Passeriformes, also can be infected. Deep, yellow, crumbly, caseous lesions often are found in advanced infections in the upper digestive tract. These abscesses can grow up to the size of ping-pong balls and, depending on where they are, can mechanically block the passage of food or respiration. Raptors feeding on birds are more likely to be infected. Nestlings of urban goshawks often carry the agent at high prevalence in their pharynx

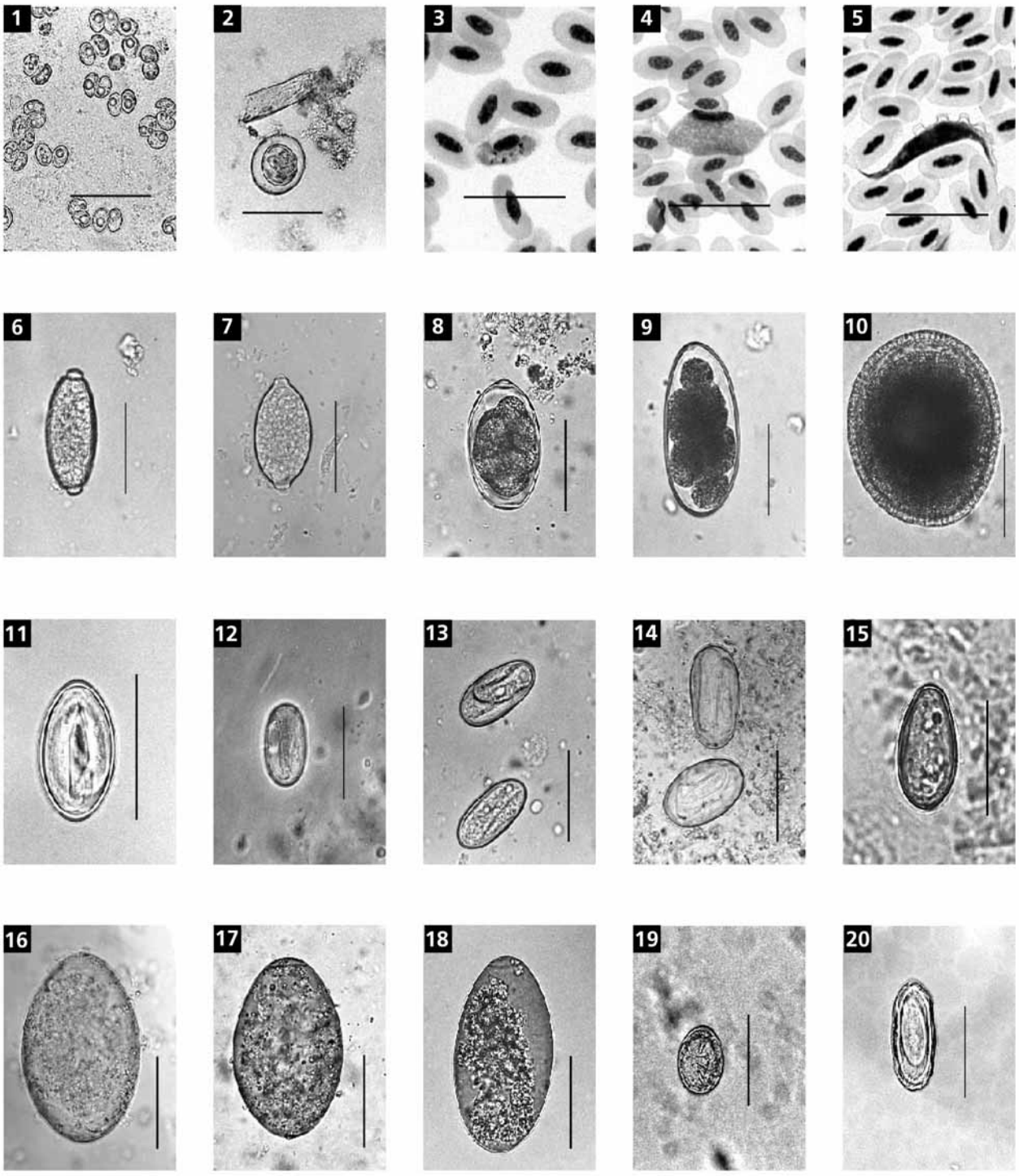

Figure 1. All protozoan parasites in line one are photographed at 1000x magnification (scale 50µm). The helminth eggs in lines two to four are photographed at 400x and 630x magnification (scale 50µm). (1) *Sarcocystis* sp., (2) *Caryospora* sp., (3) *Haemoproteus* sp., (4) *Leucocytozoon* sp., (5) *Trypanosoma avium*, (6) *Capillaria tenuissima*, (7) *Eucoleus dispar*, (8) *Syngamus trachea*, (9) *Hovorkonema variegatum*, (10) *Porrocaecum* sp., (11) *Microtetrameres cloacitectus*, (12) *Synhimantus laticeps*, (13) *Physaloptera alata*, (14) *Serratospiculum tendo*, (15) *Metorchis* sp., (16) *Nematostrigea serpens*, (17) *Strigea falconispalumbi*, (18) *Neodiplostomum attenuatum*, (19) *Cladotaenia globifera*, (20) *Centrorhynchus* sp.

without showing clinical symptoms. Sick birds may develop stomatitis (i.e., inflammation of the mucous membrane of the mouth) and have difficulty in swallowing food items. Birds often dehydrate and starve. Secondary bacterial infections can complicate and speed up the disease process.

Trypanosoma. Flagellated blood parasites of the genus *Trypanosoma* also belong to the class Zoomastigophorea. Their life cycle is indirect with the parasite being transmitted by the bite of hippoboscid flies. The pathogenicity of this genus in birds is unknown. Most diagnoses are made unintentionally by examining blood smears. Although the taxonomy is unclear, Bennett (1970) concluded that *T. avium* is the only valid species occurring in birds. Molecular parasitology should help to resolve this issue.

Sarcocystis and Ferenkelia. Coccidia of the genera *Sarcocystis* and *Frenkelia* belong to the class Sporozoea (subclass: Coccidia). These protozoans live in the mucosal layers of the intestine, where they reproduce sexually. The sporocysts excreted by the feces of the definitive host must be ingested by an intermediate host (mouse, bird). Within the intermediate host, the parasite reproduces asexually several times before cysts are built in the muscle (*Sarcocystis*) or brain (*Frenkelia*). The life cycle of the parasite is completed when a cyst in the mouse or bird is ingested by the raptor. Infections with *Sarcocystis* and *Frenkelia* spp. are seldom pathogenic. Nestlings may develop clinical symptoms such as diarrhea, feces with blood, and emaciation. Odening (1998) listed seven *Sarcocystis* spp. for the Falconiformes and four for the Strigiformes. He also declared the genus *Frenkelia* to be a synonym of *Sarcocystis* not only because of their same morphology, but also because of their developmental features.

Caryospora. The coccidia of the genus *Caryospora* (class: Sporozoea) live in the intestines of raptors. Excreted parasites are set free by the feces but need several days before reaching infectivity. The life cycle is direct, but also can involve an intermediate host. In breeding centers for birds of prey, *Caryospora* infections frequently cause problems, especially in young birds. To date, more than 14 species of *Caryospora* have been described from birds of prey (Böer 1982, Klüh 1994, Upton et al. 1990).

Leucocytozoon, Haemoproteus, Plasmodium. All three of these genera of blood parasites belong to the class Sporozoea (subclass: Coccidia). Blood-feeding insects (mosquitoes, hippoboscid flies, simulids), in which the sexual reproduction of these parasites occurs, are vectors. In the avian host, the parasites reproduce asexually in specific tissues. Only in the last stage do the parasites appear in the blood while waiting for a blood-feeding insect to infect. *Plasmodium* is more pathogenic than *Leucocytozoon* and *Haemoproteus*. *Plasmodium*, in particular, causes problems in translocated birds from areas where birds are not immunologically adapted to these parasites (e.g., Arctic, Antarctic, Himalayas). Six species of *Haemoproteus*, one species of *Leucocytozoon* and eight species of *Plasmodium* occur in falconiforms. Four species of *Haemoproteus*, nine species of *Plasmodium*, and one *Leucocytozoon* are known to occur in Strigiformes (Bennett et al. 1993, 1994; Telford et al. 1997, Valkiunas 1997).

Rare Protozoan Parasites in Raptors

Other blood parasites seldom reported are *Hepatozoon* spp. and *Haemogregarina* spp. (subclass: Coccidia), Babesia spp. (subclass: Piroplasmia), and Rickettsia-like organisms. *Toxoplasma gondii* uses a broad range of vertebrates as intermediate hosts, including, apparently, raptors (Lindsay et al. 1993).

Cawthorn (1993) reported two species of *Eimeria* (subclass: Coccidia) in Falconiformes and four species in Strigiformes, not including two new species described by Upton et al. (1990) in the latter group. A rarely reported protozoan infection of unknown origin is found in the kidneys of owls without causing inflammatory alterations. Burtscher (1966) diagnosed renal coccidiosis in three species of owls in Germany.

Helminths

Parasitic helminths are worms in the phyla Platyhelminthes and Nemathelminthes. Parasitic worms in the phylum Pentastomida are rarely found in raptors. Platyhelminthes are represented in raptors by the classes Trematoda and Cestoda. Among the Trematoda the subclass Digenea, and among the Cestoda the subclass Eucestoda, are of major interest in raptor parasitology. Nematodes belong to the class Nemathelminthes, which includes Acanthocephala.

These metazoan parasites usually are visible with the naked eye. The nematodes have a fully developed digestive system and the trematodes have an incompletely developed digestive tract. Cestodes and acanthocephalans digest material via their tegument.

Most *nematodes* (roundworms) are long, thread-formed worms that are pointed at both ends. Sexes are

separate and the females are generally larger than the males. Oviparous as well as viviparous species exist. The life cycle can be very simple (i.e., direct) or complex with intermediate and paratenic (accumulative) hosts, or both (Anderson 2000, Lee 2002).

The *acanthocephalans* (spiny-headed worms) are divided into a body (sometimes with a spinous surface) and a proboscis at the anterior end. The proboscis, which is armed with hooks, serves as an attachment organ. Sexes are separate. Eggs contain a spiny-armed larva. The developmental cycle of acanthocephalans that inhabit birds of prey is often indirect, including intermediate hosts (e.g., locusts). "Paratenic hosts," including amphibians, reptiles, and mammals, feed on locusts and accumulate the larva before the parasite reaches its definitive host.

Digenetic *trematodes* (flukes) usually are oval and dorso-ventrally flattened, with two suckers (i.e., an oral sucker surrounding the mouth, and a ventral sucker). Digenetic trematodes are mainly hermaphroditic. Exceptions include the schistosomes. Some species are capable of self-fertilization. Eggs are relatively large and always have an operculum, or cap. The life cycle of the digenetic trematodes is by far the most complex among Platyhelminthes, and also is among the most complex animals (Cheng 1986).

The *cestodes* (tapeworms) are divided into three regions: the head (scolex), the neck (proliferation zone), and the strobila (chain of proglottids). The scolex, which serves as an attachment organ, generally bears hooks and suckers. The strobila, the largest part of the cestode, is made of proglottids, the single segments which generally contain a complete hermaphrodite set of reproductive organs maturing towards the posterior end of the worm. The last proglottids are gravid (i.e., filled with eggs). The eggs contain larva (oncosphere) with three pairs of hooks. Most cestodes require an intermediate host for their development.

The *pentastomids* (tongue worms) are elongated and often segmented. Four or six rudimentary legs are present on the larvae. Adult pentastomids have two pairs of sclerotized hooks in the mouth region. Females are larger than males. The eggs contain a fully developed larva. Although the life cycle usually includes an intermediate host, the one case of a pentastomid diagnosed in a White-backed Vulture (*Gyps bengalensis*) appeared to be direct (Riley et al. 2003).

SAMPLING TECHNIQUES

Sampling techniques differ between living and dead birds. In living birds, blood, saliva, mucosal scrapings, and feces should be examined fresh and, therefore, are of better quality than those from carcasses. Interpretation of the results can be difficult as several parasites occur only in the peripheral blood or feces at some stages of development or follow a specific seasonal or daily cycle (i.e., a negative blood smear or fecal sample does not mean that the bird is not infected by the parasite). Doaster and Goater (1997) provide a good overview regarding collection and quantification techniques for avian helminths and protozoans.

Protozoa

A wet-cotton swab is used to collect saliva or mucosa from the bird's oropharynx to examine for *Trichomonas gallinae*. This swab expressed into warm water should reveal highly motile flagellate parasites when positive. Flagellates should be stained with Giemsa-solution. A more sensitive technique is to grow the parasite in a culture medium. Allowing the parasite to multiply for 3 days at 38°C and then scanning a drop of medium under a microscope is recommended. It is not possible to collect *Trichomonas* sp. from dead birds because the flagellate, which is temperature-sensitive, dies within few a minutes after the host dies.

Trypanosoma spp. often are randomly detected in classical blood smears (see blood parasites). A more reliable technique is to cultivate blood or bone marrow on blood agar. Kucera (1979) described a simple method for field diagnosis of avian trypanosomes using small penicillin bottles.

Coccidia such as *Sarcocystis* spp. or *Caryospora* spp. are diagnosed in the feces or intestinal mucosa of their definitive host. A direct smear from a fecal sample often is sufficient to find oocysts of coccidia. The standard method is flotation using a solution with a high specific gravity (i.e., saturated sugar- or NaCl-solution). A McMaster chamber can be used (see Appendix 1) to quantify the number of oocysts or helminth eggs.

Fresh fecal samples yield the highest-quality parasite stages. Fresh samples can be obtained by covering with foil the ground where the bird normally defecates. This is easily done in captive birds and also can be done in free-ranging birds with a known roosting site. During collection one should avoid the urinal part of the feces which makes the direct smear difficult to read. Uric-

acid crystals are opaque and parasite stages may be hidden. If the sample is sent by mail, an unbreakable sealed container should be used. If the transportation requires more than three days a small amount of isotonic buffered 4% formalin solution (i.e., approximately half of the volume of the sample itself) should be added to reduce bacterial growth. Often a direct smear is sufficient to diagnose parasite developmental stages, but sometimes a concentration of eggs or oocysts is needed. Simple flotation can be used to concentrate samples. It is not necessary to pass raptor fecal samples through a wiremesh filter, as they do not contain large amounts of plant matter. It is important to dissolve a small part of the sample in a saturated sugar or NaCl solution and mix it thoroughly until all large particles are broken down. It should then stand for 30 min, after which the surface film can be removed with a cover glass or a pipette for examination. The suspension also can be centrifuged to concentrate the non-floating material at the bottom, but there are many different flotation methods described in the parasite text books. For the direct-smear method a small drop of isotonic solution (RLS) helps to dilute the material so that eggs or oocysts may become easily visible. A 100 to 400x microscope is adequate to find and identify parasite stages.

Coccidian oocysts are diagnosed by their size and appearance: oocysts contain two sporocysts with four sporozoites each (Sarcocystis-type), or four sporocysts with two sporozoites each (Eimeria-type); in the Sarcocystis spp. the oocyste-membrane is very thin, giving it an appearance of a "double-egg." The genera Sarcocystis and Frenkelia (Fig. 1.1) cannot be differentiated using the oocysts which are excreted sporulated (i.e., sporocycsts and sporozoites are visible). *Caryospora* oocysts (Fig. 1.2) are much larger and are not sporulated at the time of excretion, resembling a fried egg. Their sporulated oocysts contain one sporocyst with eight sporozoites. Blood protozoa are found intracellular in erythrocytes or leucocytes, or both (Fig. 1.3-4) or in the plasma (Fig. 1.5). Identification to species in helminths is possible in only a few cases including the species *Capillaria tenuissima* (Fig. 1.6) and *Eucoleus dispar* (Fig. 1.7), both of which can be differentiated by their egg surfaces: striated in the former and dotted in the latter. Eggs in this helminth family have typical plug-like prominences at each pole. Other eggs can be determined only to the family or genus. Eggs of the family Syngamidae with *Syngamus trachea* (Fig 1.8) and *Hovorkonema variegatum* (Fig. 1.9) contain a number of blastomeres. Ascarid eggs including *Porrocaecum* sp. (Fig 1.10) have a golf-ball appearance with a dented surface that often attracts debris. Spirurid eggs (Fig. 1.11-14) are asymmetric and often contain a folded embryo. Trematode eggs (Fig. 1.15-18) are characterized by an operculum at the upper tip of the egg through which the larvae (miracidium) hatches. Most cestode eggs (Fig. 1.19) of raptors already contain a larvae with three pairs of hooks. Acanthocephalan eggs (Fig. 1.20) are embryonated with three shells, sometimes with visible hooks. A McMaster chamber (see Appendix 1) can be used to count eggs or protozoan oocysts.

Blood parasites typically are examined by the use of a blood smear. To perform a blood smear, a small amount of blood is taken from the bird, preferably with a syringe and a needle. Insulin needles with a small diameter cause a minimal lesion in the skin and vessel of the bird. The blood must be pulled and pushed slowly from the syringe so that the cells are not ruptured. A small drop of blood is placed on one end of a slide. A second slide can be used to smear the blood across the first slide. The second slide is arranged with its small edge at an angle of 45° to the first horizontal slide to allow the blood to spread along the edge. With the blood attached to the edge, the slide is pushed across the horizontal slide to create a thin blood film. It is important that the thin film tapers off on the slide with a distinct margin. The monolayer near the margin can be used to identify single blood cells and blood parasites. Air-dried blood smears should be fixed in pure methanol for one minute soon after preparation.

Helminths

Endoparasitic worms can be obtained from living birds using antihelmintics that kill or paralyze the worms, which will then be excreted in the feces within 24 hours (cf. Cooper 2002, Heidenreich 1997).

The most reliable method for collecting helminths is to dissect a bird after it has died. Carcasses from rehabilitation centers or wildlife clinics often are available for this purpose. One should have the appropriate background information on the bird (i.e., species, age, sex, circumstances of finding, location, kept in captivity, medically treated, date, name and address of finder, etc.) before starting a necropsy. This information will help to evaluate the biological data obtained from the bird. Standard dissection protocols for birds and raptors are provided by Latimer and Rakich (1994) and Cooper (2002). All internal organs should be examined completely. Most helminths are found in the digestive sys-

tem. Thus, the oropharynx, oesophagus, proventriculus, gizzard, small intestine, large intestine, bile ducts, pancreatic ducts, and cloaca should be opened longitudinally and examined. Arranging the tract in a spiral in large Petri dishes and examining it carefully at low magnification (6–60x) under a stereo-microscope helps one avoid overlooking even the smallest worms. The trachea, air sacs, and body cavity also should be scanned under a stereo-microscope. Other internal organs such as the lungs, liver with gall bladder, and kidneys should be dissected under the stereo-microscope to look for migrating larva or for parasitic cysts. Impression smears should be taken from the spleen, liver, kidneys, and lungs and stained with Giemsa solution to check for protozoan parasites. Scrapings from the mucosal layer of the intestine should be examined for the presence of oocycsts. Helminths should be handled with care so as not to destroy features important for identification. The worms can be removed gently from the attached side and washed in tap water or normal saline solution. Helminths from fresh birds should be killed in a standardized way. Nematodes and acanthocephala can be heated carefully in a glycerin (5%):ethanol (70%) solution to prevent contraction. Trematodes and cestodes can be relaxed in a refrigerator prior to fixation. Trematodes should be fixed in a Bouin-solution (see Appendix 1) for 24 hours and cestodes can be killed and fixed in a 10% formalin-solution (neutral buffered) and then are stored in a glycerine (5%):ethanol (70%) solution. Use of these solutions helps to identify the parasite using morphological features. They are not appropriate for a genetic analysis. For this purpose, specimens should be conserved in pure ethanol or frozen until DNA analysis is possible.

IDENTIFICATION TECHNIQUES

To identify protozoan parasites it may be necessary to stain them or to allow further development (e.g., sporulation). Methanol fixed-blood smears are stained in Giemsa-solution for 20 to 30 minutes (see Appendix 1). After staining, the remains of the staining solution are washed away with tap water and flushed in aqua dest. After air-drying, the blood smears are examined microscopically at 25, 100, 400, and 1000x magnification. Some coccidian parasites are excreted unsporulated (e.g., *Caryospora*, *Eimeria*). To enable sporulation small fecal sample or a mucosal scrape is given on a slide together with a drop of water and covered with a cover slip. This preparation is then placed in a Petri dish together with a moistened piece of pulp and kept for 24 to 48 (72) hours at room temperature. The identification is then performed using the literature listed above.

The classical identification of helminths is based on morphological features such as body size, buccal capsule, spicules, ornaments, suckers, testes, cirrus, hooks, probosces, etc. Internal structures needed for identification become visible by passing the nematodes and acanthocephala through a lactophenol-mixture (20 g crystalline phenol, 20 ml lactic acid, 10 ml glycerin, 20 ml aqua dest) or a lactoglycerol-mixture (equal parts of lactic acid, glycerol and distilled water) for some minutes. Trematodes and cestodes need to be stained for identification. After fixing, trematodes should be squeezed between two slides and stained with an alum-carmine-solution (see Appendix 1). To stain the trematodes, picric acid needs to be washed out with 70% ethanol for about 24 hours. The specimens then need to be washed in aqua dest, followed by staining with alum-carmine solution for 10 to 60 minutes, and then washed in aqua dest again. An alternative staining method using Gower's acetic carmine is described by Schell (1970). Dehydrate the specimen sequentially in 60%, 70%, 80%, 96% ethanol for 3 to 10 minutes in each concentration. Then wash the cestodes in pure n- or isopropanol for 3 to 10 minutes. Next clear them in xylene for 10 to 15 minutes and mount them in Canada balsam prior to identification. The cestodes are stained for eight minutes in a hydrochloric acid-carmine solution (See Appendix 1) and then transferred into a 1% hydrochloric-acid ethanol solution. Depending on the quality of the cestodes, color changes occur within 30 minutes. The cestodes are then washed in 60% ethanol and moved through a series of higher concentrated alcohols for dehydration starting with 70% ethanol for 24 hours followed by 96% ethanol for 24 hours. Finally, the specimens are washed in pure propanol for 10 to 15 minutes and cleared in xylol and mounted in Canada balsam. Schmidt (1986) also described a staining method using hematoxylin. All helminths are microscopically examined at 25, 100, 400, and 1000x magnification. Using internal (and external) structures for identification requires some experience.

Useful identification guides for helminths to the family or genus level (very rarely to species level) are uncommon and sometimes appear in languages other than English. The list of references below should be useful. Identifying nematodes can be accomplished using keys provided by Skrjabin (1953, 1957, 1963,

1964, 1965, 1967, 1968), Anderson et al. (1974a,b, 1975a,b, 1976a,b, 1978a,b, 1980a,b, 1982), Hartwich (1975, 1994), and Anderson and Chabaud (1983). Acanthocephala can be identified using Chochlova (1986). Trematodes can be identified using Skrjabin (1950, 1959, 1960, 1971), Dubois (1968, 1970), Gibson et al. (2002), and Jones et al. (2004). Cestodes can be identified using Abuladze (1964), Chertkova and Kosupko (1978), Schmidt (1986), and Khalil et al. (1994). To identify rare endoparasites it often is necessary to read the original or, when available, revised species descriptions. Doing so often requires an extensive literature search.

MOLECULAR PARASITOLOGY

Molecular parasitology is a new and fast evolving discipline. The tools described below represent a small selection of those available. Molecular-biology techniques, including DNA sequencing, can be useful in identifying species as well as in answering questions of systematics (Gasser 2001). To understand the mechanisms of parasite origin, phylogenetic studies are needed and correct identification of specimens is a prerequisite (Blaxter 2001).

Parasitic protozoa may be identified by comparing sequences of the internal transcribed spacer region 1 (ITS-1) of the ribosomal RNA (Marsh et al. 1999) or the 18S small subunit (SSU) of the ribosomal DNA (Jenkins et al. 1999). The PCR protocol of Bensch et al. (2000), as modified by Hellgren et al. (2004) and Waldenström et al. (2004), can be used to amplify sequences of the cytochrome b gene of the avian blood-parasites *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*.

Different molecular markers can be used to study nematodes. Slowly evolving genes such as cytochrome c, globin, RNA II polymerase, and heat shock protein 70, are useful in this regard at higher taxonomic levels (i.e., Order or higher). The ribosomal DNA contains several conservative coding sequences including SSU, 28S or large subunit (LSU) and 5.8S, and highly variable non-coding sequences ITS-1 and ITS-2 (Blaxter 2001). The conservative 5.8S sequence is suitable for phylogenetic studies at the level of Order or higher (Chilton et al. 1997). The ITS sequences are useful for genus or subfamily levels (Chilton et al. 2001, Morales-Hojas et al. 2001).

The cytochrome c oxidase gene I (COI gene) can be used to differentiate some types of trematodes (Wongratanacheewin et al. 2001, Pauly et al. 2003) and cestodes (Bowles and McManus 1994) at genus or species level. The 3' end of the ITS-1 element can be used in elucidating phylogenetic relationships of distinct taxa (Schulenburg et al. 1999), and the full ITS-1 sequence is useful for differentiating trematodes at the species level.

Because they provide more sensitive tools, such as detecting low parasite burdens with specific markers, molecular methods will help achieve deeper insights into parasite diversity by detecting morphologically undistinguishable (i.e., cryptic) species. As a result, some infections of protozoic and metazoic parasites will be more easily diagnosed, because they are often overlooked with classical methods, including blood parasite infections detected with blood smears.

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Appendix 1. Recipes for solutions and the McMaster chamber mentioned in the text.

Acid-carmine solution	Boil 4 g carmine, 15 ml aqua dest, and 1.5 ml concentrate hydrochloric acid using a Liebig cooler. After cooling add 85 ml 95% ethanol.
Alum carmine solution	Boil 5 g potassium-aluminium-sulfate, 2 g carmine, and 100 ml aqua dest for 1 hour. When cool, filter the solution and add some thymol crystals for preservation. Store the solution in a refrigerator.
Bouin-solution	Mix one part 40% formalin plus three parts aqua dest filled with picric acid until saturation. Add one part glacial acetic acid to 10 parts of this stock solution.
Giemsa-solution	Mix 10 ml Giemsa with 190 ml distilled aqua dest buffered to pH 7.2 for 10 minutes at 40°C.
McMaster Chamber	The specific slide made of glass or plastic can be used to count parasite eggs or oocysts of protozoa per gram of feces. This standard method is often described in classical text books of parasitology, but information also can be obtained from the homepage of the Food and Agriculture Organization of the United Nations (FAO): www.fao.org/ag/AGAInfo/resources/documents/Parasitology/EggCount/Purpose.htm (last accessed 17 August 2006).