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

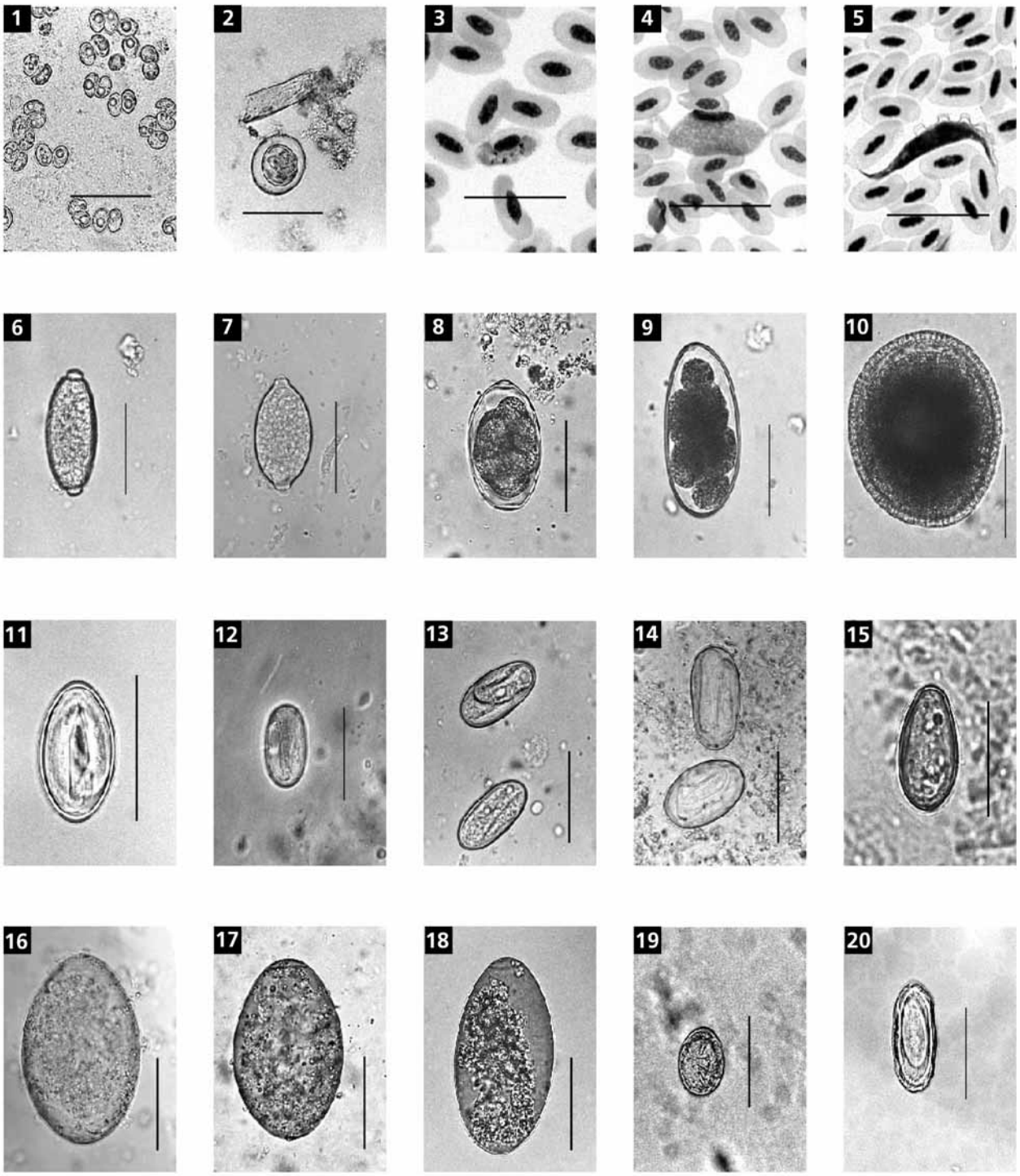

Figure 1. All protozoan parasites in line one are photographed at 1000x magnification (scale 50μm). The helminth eggs in lines two to four are photographed at 400x and 630x magnification (scale 50μm). (1) *Sarcocystis* sp., (2) *Caryospora* sp., (3) *Haemoproteus* sp., (4) *Leucocytozoon* sp., (5) *Trypanosoma 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).