Parasitic Diseases of Wild Birds

Edited by
Carter T. Atkinson, Nancy J. Thomas & D. Bruce Hunter

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Preface

More that 30 years ago, John W. Davis, Roy C. Anderson, Lars Karstad, and Daniel O. Trainer edited the first edition of *Infectious and Parasitic Diseases of Wild Birds*. Since then there has been an explosion of new knowledge about parasitic diseases of wild birds, as wildlife disease specialists, ecologists, and evolutionary biologists have continued to unravel how parasitic protozoans, helminths, and ectoparasites affect wildlife populations. We continue in the footsteps of the first editors of this work by significantly expanding and updating the parasite portion of their original book. This work is a companion volume to *Infectious Diseases of Wild Birds*, which was published in 2007 by Blackwell Publishing, and complements *Infectious Diseases of Wild Mammals*, 3rd edition, edited by Elizabeth S. Williams and Ian K. Barker, and *Parasitic Diseases of Wild Mammals*, 2nd edition, edited by William M. Samuel, Margo J. Pybus, and A. Alan Kocan (Iowa State University Press). Taken together, these four volumes provide an important source of reference material for biologists and wildlife mangers, wildlife and veterinary students, professionals in the fields of animal health and wildlife disease, and evolutionary biologists with interests in disease ecology. We gratefully acknowledge our colleagues who established such excellent models for us to follow.

This book focuses on the disease conditions produced by parasitic protozoans, helminths, leeches, and ectoparasitic arthropods, e.g. mites, and biting flies in free-living wild birds. Unlike most parasitology texts, this book emphasizes effects on the host rather than the parasites themselves, but still includes important information about their etiology, life cycles, transmission, and diagnosis. While no single work can cover the entire spectrum of wildlife parasites, we have attempted to assemble chapters that are both specific (e.g., Chapter 9, Disseminated Visceral Coccidiosis in Cranes) and general (e.g., Chapter 14, Cestodes) in their treatment of some of the diverse groups of organisms that use wild birds as intermediate or definitive hosts. In all cases, we have urged authors to avoid generalities and include specific examples of host–parasite associations that can lead to clinical disease. We owe a great debt to the authors of these chapters both for their expertise in the material and for their willingness to endure the inevitable delays and revisions that are inherent in multiauthored works.

Each chapter provides a classical description of the history, effects on the host, and causative agent, but the authors were also challenged to provide perspectives on the significance of the disease to wild birds and to document population impacts, an aspect that is particularly difficult to quantify in the wild. Unlike other volumes in this series, we elected to begin this book with an introductory chapter by Gary A. Wobeser who discusses some of the costs and effects of parasitism in wild avian populations. This chapter provides a succinct discussion of some of the difficulties in assessing impacts of parasitism on wild birds and provides a good framework for assimilating the detailed information in the sections that follow.

We used *The Clements Checklist of Birds of the World*, 6th edition (Cornell University Press, 2007), as the authority for avian nomenclature and elected to allow authors to make individual decisions about whether to follow the proposed standardized nomenclature for parasitic diseases (SNOPAD; http://www.waavp.org/node/40). As a result, some chapters follow this terminology (e.g., Chapter 4, Leucocytozoonosis) while others retain the more traditional terminology (e.g., Chapter 7, *Histomonas*). Because many unpublished data on wild bird diseases have been compiled in laboratory and diagnostic files, citations of unpublished data were allowed for repositories of large, permanent, accessible institutions, such as the Canadian Cooperative Wildlife Health Centre, U.S. Geological Survey National Wildlife Health Center, and Southeastern Cooperative Wildlife Disease Study.

Grateful acknowledgment goes to the Iowa State University Press, which guided this project through its initial stages, and to Blackwell Publishing, which took it over and shepherded it through to completion. We owe sincere debts of gratitude to Donald J. Forrester who was instrumental in the initial organization of the book and to Amy Miller for her significant

contribution in the technical editing of the final manuscript. We acknowledge the support of the U.S. Geological Survey, Wildlife and Terrestrial Resources Program, and the University of Guelph. This book is dedicated to the Wildlife Disease Association, whose members initiated the revision of this book series and who continue to provide the backbone of growing knowledge in the field of wildlife disease. Royalties that accrue from sales of this book will be provided to the Wildlife Disease Association.

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Section I:
Introduction

1
Parasitism: Costs and Effects

Gary A. Wobeser

Parasitism has been defined in many ways, but in terms of wildlife disease, it is usually taken to mean an obligatory trophic association between individuals of two species in which one (the parasite) derives its food from a living organism of the other species (the host). An individual host bird can be viewed as an island of habitat that provides resources for parasites, with the parasites deriving benefits while the host is harmed or bears some cost. Parasitism is common in nature; for example, Price (1980) estimated that half of all animal taxa are parasitic. Parasitism is ubiquitous in wild birds and individual birds are affected by many different parasites during their lifetime, but our understanding of the parasites that occur in wild birds is fragmentary.

Moore and Clayton (1997) concluded that the majority of parasites of wild birds have yet to be described taxonomically. Some groups, such as blood-inhabiting protozoa (the hematozoa), have been studied widely, perhaps because of the ease with which blood can be collected from living birds, while little is known about other groups such as intestinal flagellates. But even within the hematozoa, species diversity has probably been greatly underestimated (Bensch et al. 2007). Similarly, more is known about the effects of arthropod ectoparasites than about the effect of protozoa and helminths on birds, and cavity nesting birds have been studied more extensively than most other species because of the relative ease in capturing, examining, and following these birds.

Studying parasitism in wild birds is subject to a number of constraints that make working with disease in any free-ranging species more difficult than studying humans or domestic animals. These include:

- inadequate baseline information about the host species. Knowledge of avian life history traits is rudimentary (Zera and Harshman 2001), and so one often must extrapolate from other species and collect information about the basic biology of the host while trying to understand a host–parasite relationship;
- difficulty in quantifying factors related to disease. It is impossible to assess the significance of a parasite for a population without the ability to calculate basic epidemiological proportions such as prevalence, incidence, morbidity, and mortality rates. The number of individuals affected by a parasite (the numerator for such calculations) is usually difficult to determine and the population at risk (the denominator) rarely can be measured adequately;
- the need to consider the long-term effect of a parasite in wild birds. This may be very difficult, even when the number affected and the population at risk can be determined. If a disease, such as coccidiosis, occurs in a flock of chickens and 15% die, the significance of the disease is that 15% fewer chickens go to market. However, a similar 15% loss in a wild bird population might result in more resources per capita for the remaining birds, leading to reduced mortality from other factors and/or improved reproduction. The potential for compensation or other delayed effects may be very important in assessing the impact of a parasite on wild birds at the population level;
- the sample of wild birds available for study is usually biased by the method of collection and may not represent the actual state of nature. Depending on the method of collection, affected birds may be under- or overrepresented, even in groups collected by mass-capture methods (Sulzbach and Cooke 1978); and
- the anonymity of wild birds, except for the small number marked by the researcher. For instance, although age is an important disease determinant, the age of wild birds often cannot be determined except to differentiate hatch-year from after-hatch-year birds. Individuals seldom can be traced back in time to discover previous exposure to disease agents or forward in time to discover

their fate. Commonly used techniques such as retrospective and prospective case–control studies that are useful in human and veterinary epidemiology are impossible except in unusual circumstances, such as in birds with a high degree of nest site fidelity.

A fundamental feature of parasitism is that the presence of a parasite involves a cost to the host. The costs of parasitism may include:

- loss of resources extracted by the parasite directly from the host, for example, loss of blood to blood-feeding ectoparasites;
- competition between the parasite and the host for resources, as occurs with cestodes that absorb nutrients from the host's gut content;
- costs to the host for defense against parasites. These may include foregoing resource-rich areas to avoid areas where parasites may be present, costs for grooming, moving away from parasites, or abandoning a nest, costs to develop and maintain innate and acquired resistance, and costs to activate these systems;
- costs resulting from tissue injury related to the parasite. This may be direct damage caused by the parasite or, more often, injury from the inflammatory and immune response to the parasite. Some injuries may result in dysfunction, such as reduced mobility, reduced digestive efficiency, or increased loss of nutrients through intestinal or kidney injury, that interfere with obtaining or retaining resources;
- costs related to improper development as a result of parasitism early in life (e.g., Spencer et al. 2005); and
- costs to repair or replace damaged tissues.

The diversity of parasites and the variety of ways that they interact with hosts make it difficult to measure the cost of a single parasite species; to compare the relative cost of different parasites such as the lice, intestinal coccidia, and tracheal worms, all of which might be infecting a single host; or to understand how these parasites may interact with each other and with other environmental factors to affect a host population. The costs described above are related to resources, and particularly to energy ["*the single common denominator of life*"; "*something that is absolutely essential and involved in every action large or small*" (Odum 1993)]. Energy is a measure of the ability to do work and is a "currency" that can be used to consider the costs of all types of parasitism, at least conceptually if not quantitatively at this time. Four basic features must be considered when using energy as a currency to consider parasitism:

- The supply of energy is limited. Most birds are unable to increase their intake of energy readily, and so they must function within a finite budget. In other words, a bird cannot use more energy than it can assimilate or has in storage.
- The amount of energy available and accessible is not constant or uniform. The energy available to a bird varies with the time of year, weather, habitat conditions, and the number of competitors for that energy. Not all individuals in a population have equal access to the resources that are available; thus, within a group or population some birds may have abundant resources while others do not.
- Use of energy for one purpose reduces the amount available for other uses. Most of the energy assimilated by a bird is used for maintenance, that is, keeping the body functioning, repaired, maintaining a high core temperature, avoiding predators, and defending against disease. Energy that remains can be used for production (growth and reproduction) or stored as fat for future use. If extra energy is used to defend against parasites or to repair tissue injured by parasites, the energy available for reproduction or growth is reduced. For instance, the cost of producing antibody to a novel antigen is equivalent to that of producing half an egg in female House Sparrows (*Passer domesticus*) (Martin et al. 2003) and mounting an immune response resulted in asymmetry of flight feathers in nestling Mountain Chickadees (*Poecile gambeli*) (Whitaker and Fair 2002). Conversely, increased reproductive effort may result in reduced ability to mount a defense against parasites (Deerenberg et al. 1997).
- The need for energy for various purposes is highly variable among individuals and at different times of year.

Because an individual cannot maximize all life history traits simultaneously, life history theory suggests that a bird should adopt a strategy that optimizes energy use among resource-demanding activities, such as defense and reproduction, to maximize lifetime fitness. Ecologists use the term "trade-off" for this process of making physiological choices among competing needs for resources that should maximize the chances of an individual's genes being passed on to the next generation. Individuals that make the wrong choices are less successful or "fit," and this may provide a basis for genetic selection.

As a result of heterogeneity in both the supply of energy and the need for energy, the appropriate physiological trade-offs in relation to parasitism vary among individual birds and for different parasites, and the pattern of trade-offs is different seasonally and annually. For this reason, the reaction to parasites and the effects of parasitism must always be considered in terms of the context in which parasitism is occurring and of how the situation might influence resource trade-offs. For instance, during one season a bird may be in poor nutritional condition and need to direct all its available resources to simply staying alive, with little or no ability to mount an effective defense against parasites or to grow or reproduce. At another time of year the same bird may have ample resources to meet all needs, and so it can afford strong resistance to parasites and still be able to grow and reproduce effectively.

Young birds may have different priorities than adults and the sexes may have different strategies and trade-offs. For instance, Tschirren et al. (2003) suggested that a greater need for carotenoid-based coloration for signaling by male Great Tits (*Parus major*) might lead to a trade-off that results in reduced immunocompetence in males. Privileged individuals within the population, such as birds that possess a territory, may have a totally different context for trade-offs related to parasites than do the "have-nots" within the population. Changes in environmental conditions may change the context; for example, Blow Fly (*Protocalliphora braueri*) larvae had no effect on Sage Thrasher (*Oreoscoptes montanus*) nestling weight, size at fledging, or mean fledgling age, but in a year with cold wet weather, survival and fledging success were markedly reduced among parasitized birds compared to unparasitized birds (Howe 1992).

Knowledge of how trade-offs occur in relation to parasitism is fragmentary at this time and general rules about which activity (reproduction, growth, defense against predators or parasites) should take precedence for resources are likely subject to many exceptions. For instance, hosts may be selected to develop acquired immunity to only some of the disease agents that they encounter (Boots and Bowers 2004). While mounting a strong defensive response to parasites is likely a "good" thing generally, in some situations it may be adaptive to suppress the defensive response. This may be the case in nesting Common Eiders (*Somateria mollissima*). Female eiders do not feed during breeding and face severe resource restrictions while incubating. Birds that do not begin with adequate resources abandon their nest in order to survive.

Hanssen et al. (2004) immunized incubating female eiders with nonpathogenic antigens, including sheep red blood cells. Not surprisingly, the rate of successful immunization was not very good compared to what would be expected at other times of year. Under these circumstances, it appears that the appropriate choice for many eiders is to use their limited resources to survive and reproduce rather than to mount an immune response. A second part of the same study compared survival of birds that mounted an immune response to that of birds that did not produce antibodies. Both responding and nonresponding eiders had sufficient resources to complete reproduction; however, only about 27% of birds that produced antibody to sheep red blood cells returned to the colony in subsequent years, compared with approximately 72% of birds that did *not* produce antibody. Under these conditions, females that invested in an immune response "*experienced considerably impaired long-term survival*" compared to females that did not respond. This example also serves to illustrate that the effect of a trade-off on fitness may be delayed.

The cost to the host is not obvious for most parasites encountered in wild birds. It is only in a minority of situations, described elsewhere in this book, that parasitism is clearly associated with recognizable functional impairment of the host that we can characterize as disease. The apparently "benign" nature of many parasites could be because:

- the effect of the parasites actually is so trivial as to be undetectable;
- the cost is not trivial but it is tolerable; that is, the bird has sufficient resources to cover the costs without significant negative effects on other functions *under conditions at the time the effect was measured*;
- the cost of parasitism is obscured by other more proximate regulatory factors such as predation and competition. Predation is thought to be a major factor in shaping the life history of birds (Zera and Harshman 2001) and parasitized prey may be taken disproportionately by predators (Temple 1987). In some situations the parasite benefits if the infected host is eaten by an appropriate predator (parasite-induced trophic transmission; Lafferty 1999). But infections in which there is no apparent benefit to the parasite may make animals more susceptible to predators, perhaps because of the pathology induced by the parasite. Hudson et al. (1992a) found that Red Grouse (*Lagopus lagopus scotica*) killed by predators were more heavily parasitized by the cecal nematode (*Trichostrongylus tenuis*) than were hunter-killed birds and that birds with many worms may emit more scent and, hence, be more vulnerable to mammalian predators. In some situations, increased vulnerability to predators may be related to energy trade-offs and reduced resources for

predator vigilance or avoidance. For instance, Common Redshanks (*Tringa totanus*) that are energetically stressed (as might result from parasitism) respond by taking risks that increase the probability of predation (Quinn and Cresswell 2004). The interaction between predation and parasitism is undoubtedly complex. Navarro et al. (2004) found that House Sparrows exposed to potential predators (cat or owl) had reduced T-cell-mediated immune response and a higher prevalence and intensity of infection with *Haemoproteus* spp. than did sparrows exposed to nonthreatening animals (rabbit or pigeon), suggesting that even the threat of predation may alter trade-offs that influence parasitism. Although little is known about the effect of parasitism on intraspecific competition, this may be an important factor. For instance, male Greater Sage-Grouse (*Centrocercus urophasianus*) infested with lice are discriminated against for breeding (Spurrier et al. 1991). Females appear to recognize infected males by the occurrence of petechial hemorrhages on the air sacs and males infested with lice are shunned, and so their reproductive input to the population is minimal; that is, their fitness is very low and there is likely negative selection against their genotype. In a similar manner, male Red Grouse infected with *T. tenuis* may have difficulty defending a territory (Delahay et al. 1995). Consideration of interactions between parasitism and competition must also include competition among species that share parasites, such as the Ring-necked Pheasant (*Phasianus colchicus*) and Gray Partridge (*Perdix perdix*) that share *Heterakis gallinarum,* with asymmetrically severe effects on the partridge (Tompkins et al. 2001b); and

- the cost is not trivial but it goes undetected because of insensitivity of the methods used to look for effects. For instance, it would be very easy to dismiss the tiny hemorrhages caused by lice as inconsequential to male Greater Sage-Grouse, without even considering that they might have a profound effect on behavior, reproductive success, and natural selection. The costs of parasitism could also be overlooked because the wrong individuals within the population are examined, the interaction between parasite and host is examined in an inappropriate context (e.g., at the wrong time of year or in an experimental situation in which resources are not limited), inappropriate parameters are measured, or because the long-term (lifetime) consequences of parasitism are not measured. Møller (1994) suggested that the cost of parasitism in nestling birds could be paid by the nestlings through reduced growth or survival or by the parents through reduced survival or future reproductive success as a result of having to provide additional resources to the parasitized young. Bize et al. (2003) found that nestling Alpine Swifts (*Tachymarptis melba*) can compensate for early growth retardation by rapid feather growth, so that if measured at fledging no effect might be obvious; however, rapid feather growth may result in poor feather quality with later effects (Dawson et al. 2000). Nutrient shortage in early development can have other serious long-term consequences including effects on adult dominance rank, morphology, and lifespan (Metcalfe and Monaghan 2001). Island Canaries (*Serinus canaria*) infected with plasmodia as nestlings have structural changes in their brain and reduced song repertoire as adults (Spencer et al. 2005). The effects of parasites are usually not distributed evenly or fairly among all members of a population, which complicates measuring their cost. Metazoa characteristically are distributed in an aggregated manner within the host population (Shaw et al. 1998). Most hosts have few or no parasites and a few individuals have many parasites (often referred to as the 20:80 rule: 20% of the population carries 80% of the parasites). Severe effects are likely to be confined to those individuals with many parasites. Measures of central tendency, such as average intensity of infection and average cost of parasitism, may not be helpful in understanding the significance of the parasite if effects are concentrated in a small group of heavily infected individuals. These animals at the extreme end of the distribution are also important as the major source of infection within the population, but samples drawn from the population are unlikely to contain these individuals unless the sample is very large. Much of the information available on the occurrence of parasites in wild birds comes from the study of birds that died of other causes, because it is inappropriate to kill large samples of birds simply to record their parasites. At one extreme, such a sample may primarily consist of the survivors of conditions that were severe, resulting in underestimation of the cost of parasitism. At the opposite extreme, the sample may contain the few significantly affected individuals in the population, and so the cost to the population is overestimated.

"*While the study of specific host–parasite relationships have proven insightful, they reflect only a small part of the wealth of parasites and pathogens in an*

animal's internal and external environment" (Lochmiller and Deerenberg 2000). Virtually all the information available about parasites of birds relates to the effects of individual parasite species, but individual birds are host to many different parasites, often simultaneously; for example, a single feather may be infested with 6 species of feather mite (Pérez and Atyeo 1984) and a group of 45 Lesser Scaup (*Aythya affinis*) were infected by almost 1 million individuals of 52 different helminth species (Bush and Holmes 1986). Examining the effect of parasitism as the interaction between two species fails to account for interactions among parasites that might be additive, synergistic, or antagonistic. Almost nothing is known about the effects or dynamics of parasite assemblages or communities in wild birds.

The largest challenge for those interested in parasites of birds is to answer the question "Do parasites influence bird populations?" Most ecologists and wildlife managers have assumed that the answer is "No" (Tompkins et al. 2001a), but modeling suggests that parasites could regulate host populations if they reduce host survival and/or fecundity in a density-dependent manner (Anderson and May 1978; May and Anderson 1978). To understand the effect of a parasite on the host population, one needs to understand the effect of the parasite on the individual host, the prevalence and intensity of parasite infection within the host population, and the context within which the interaction is occurring. Parasites rarely result in obvious piles of dead birds but many studies have concentrated on the direct effect of parasites on mortality although "*. . . highly pathogenic parasites tend not to have an impact at the population level. . . .*" (Hudson and Dobson 1997), because this type of parasite may kill the host rapidly, thus limiting transmission to other individuals. Sublethal effects of chronic infections that are mediated through reduced fecundity are more likely to have an effect at the population level.

Much of the information available about parasites in birds is descriptive. More than 70 years ago, Aldo Leopold recognized that observational and correlational studies have limited ability to lead to an understanding of disease in wild species (Leopold 1933). Marzal et al. (2005) observed that knowledge of causal relationships of disease caused by parasites of birds "*is still rudimentary due to a scarcity of experimental manipulation*," and Tompkins and Begon (2000) stated that "*regulation by parasites can be established only by experimentally perturbing host/parasite systems away from their equilibrium levels and monitoring subsequent changes in both parasite and host densities relative to control*." Studies that include intervention through treatment of parasites in natural populations, such as by Hudson et al. (1992b, 1998) (*T. tenuis* and Red Grouse), Merino et al. (2000) (hematazoa in Eurasian Blue Tits, *Cyanistes caeruleus*), Hoodless et al. (2002) (ticks and Ring-necked Pheasants) and Marzal et al. (2005) (*Haemoproteus prognei* in House Martins, *Delichon urbicum*), and through experimental infection (e.g., Spencer et al. 2005), have provided insights into parasitism that would be unattainable with traditional observational study. As in all aspects of the study of parasitism, it is important to consider the long-term effects of such interventions. For instance, Hanssen et al. (2003) studied the effect of antiparasite treatment on nesting female eiders. There was no effect of treatment on nest success or on the survival to the next year of birds that nested successfully. However, among the females that were unsuccessful in nesting, 69% of treated birds survived compared with 18% of untreated birds. This suggests that birds that nested successfully were able to tolerate the effects of parasitism, while unsuccessful females were less able to bear the costs from parasites, resulting in a delayed effect on survival. In another example, McCutchan et al. (2004) found that a vaccine significantly protected canaries against natural infection with *Plasmodium relictum* in the year of vaccination. In the following year, survivors in the vaccinated group suffered much higher mortality than unvaccinated birds that had survived exposure in year 1, presumably because vaccine-induced immunity prevented acquisition of protective natural immunity.

Wild birds have developed a suite of trade-offs that allow them to be successful under a particular set of conditions. Environmental cues, such as photoperiod, may guide the timing of these trade-offs. Our world is changing rapidly and dramatically, especially for many wild species. With rapid anthropogenic alterations, such as climate change and environmental contamination, cues that were reliable may no longer be associated with adaptive outcomes (Schlaepfer et al. 2002). If birds are trapped by their evolutionary response to cues, they may find themselves equipped with attributes that are no longer optimal. Schlaepfer et al. (2002) used the term "*evolutionary trap*" for decisions that are now maladaptive because of a sudden anthropogenic disruption. For instance, the optimal time for reproduction by seasonally breeding birds matches peak food supply with peak nestling demand. If birds schedule reproduction based on photoperiod while food supply is determined by temperature, a mismatch in timing may result in peak nestling demand occurring while food supplies are declining, with serious consequences for fitness (e.g., Thomas et al. 2001). The effect of this type of evolutionary trap on parasitism has not been explored, but mismatches between the phenology of parasites or disease vectors and birds, as well as range expansion by parasites as a result of

climate change and interactions among parasites and contaminants, could result in parasites assuming different or greater significance in altered environments.

In summary, although parasitism is a universal phenomenon in wild birds and many parasites have been observed and described, the information is still fragmentary and largely descriptive in nature. Little is known about the effect of most parasites on their hosts and almost nothing is known about interactions among the parasites that make up parasite assemblages or communities. The cost of parasites to their hosts is difficult to measure, but using energy as a currency may be a fruitful way to understand how costs are incurred, why birds must make trade-offs that influence both their exposure and resistance to parasites, and how being parasitized may affect basic life history traits including reproduction and susceptibility to predation. Parasitism can never be considered in isolation; it must always be considered in terms of the context in which it is occurring and this consideration must include the potential effects of anthropogenic changes.

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Section II:
Protozoa

2
Haemoproteus

Carter T. Atkinson

INTRODUCTION

The species of *Haemoproteus* that infect birds are vector-transmitted intraerythrocytic parasites closely allied to the true malarial parasites of vertebrates. Unlike their close relatives in the genus *Plasmodium*, they undergo asexual reproduction or merogony within tissues rather than circulating erythrocytes. They are some of the most common and widespread blood parasites of wild birds, yet their potential significance as disease agents in wild bird populations is largely unknown. They are receiving increasing attention by avian ecologists as models for testing evolutionary theories about effects of disease on host fitness and sexual selection, but these efforts have been hampered by lack of basic knowledge about their life cycles, vectors, and epizootiology.

Some species of *Haemoproteus* can be highly pathogenic and cause severe myositis in avian hosts, but well-documented cases are still rare. These include reports of disease associated with developing tissue stages in Northern Bobwhite (*Colinus virginianus*) (Gardiner et al. 1984; Cardona et al. 2002), Luzon Bleeding-heart (*Gallicolumba luzonica*) (Earle et al. 1993), Rock Pigeons (*Columba livia*) (Farmer 1965), House Sparrows (*Passer domesticus biblicus*) (Paperna and Gil 2003), Blossom-headed Parakeets (*Psittacula roseata*) (Miltgen et al. 1981), and Wild Turkeys (*Meleagris gallopavo*) (Atkinson and Forrester 1987). Some of these reports reflect abnormal host–parasite associations, where susceptible hosts were moved outside of their natural ranges and exposed to haemoproteid parasites from closely related host species.

SYNONYMS

Haemosporidiosis. Infection with avian species of *Haemoproteus* is sometimes referred to as avian malaria, particularly in the recent ecological literature, but distinctive life history characteristics clearly distinguish them from the true malarial parasites in the genus *Plasmodium* (Valkiūnas et al. 2005).

HISTORY

The species of *Haemoproteus* that infect birds were first observed on unstained blood smears along with other intraerythrocytic hemosporidian parasites by the Russian zoologist V. Ya. Danilewsky as "... clear, colorless, transparent vacuoles, variable in shape and size, in which are present several refractile glossy-black granules" (cited in Hewitt 1940). With the advent of Giemsa staining to differentiate parasites from host cells (Garnham 1966), the diversity and broad host range of these parasites became evident, but their host specificity, life cycles, and vectors were not known. Considerable confusion existed as to what comprised a species, and the taxonomy of this group has been in a continual state of flux for over a hundred years.

Major historical milestones over the past century include discovery that *Haemoproteus columbae* of pigeons and doves can be transmitted by the bite of ectoparasitic hippoboscid flies (Sergent and Sergent 1906) and the discovery that ceratopogonid flies in the genus *Culicoides* can transmit other species of *Haemoproteus* (Fallis and Wood 1957). Early recognition of the sexual stages of *Haemoproteus* (MacCallum 1898), the hippoboscid vectors (Sergent and Sergent 1906), and preerythrocytic tissue stages of *H. columbae* (Aragão 1908a) led to a number of classic investigations of the sporogonic or asexual stages of the parasite within the invertebrate vector and the preerythrocytic development of *H. columbae* within the avian host (Acton and Knowles 1914; Adie 1915, 1924; Coatney 1933). These formed an important framework during the first two decades of the twentieth century for understanding the life cycles and development of closely related haemosporidia in the genera *Plasmodium* and *Leucocytozoon*.

The vast bulk of published studies on avian species of *Haemoproteus* over the past 50 years have been surveys and taxonomic descriptions by parasitologists and disease workers. It is only in the past few years that there has been a renaissance in interest in these parasites by avian ecologists and evolutionary biologists

because ease of sampling wild birds by noninvasive blood collection makes them potentially good models for testing evolutionary hypotheses. The role that these parasites may play as pathogens in wild birds has been speculated about since their discovery, but it is only in the past 20 years that clear evidence that they can have some measurable effects on host survival and reproduction has accumulated.

DISTRIBUTION

Avian haemoproteids have a worldwide distribution in temperate and tropical climates. This wide distribution is most likely a function of the diverse habitats occupied by their ceratopogonid and hippoboscid vectors (Greiner et al. 1975). Haemoproteids have been recorded from most parts of the globe where hippoboscid and ceratopogonid vectors occur, including remote islands in the central Pacific (Work and Raymeyer 1996; Padilla et al. 2004). The greatest diversity of species occurs in the Holarctic, Ethiopian, and Oriental zoogeographic regions, with fewer numbers of species recorded from both the Neotropical and Australian regions (Valkiūnas 2005). In both North and South America, haemoproteids tend to have a relatively uniform distribution across the continent and are virtually absent in the high arctic tundra, most likely because of the absence of suitable vectors (Greiner et al. 1975; White et al. 1978; Bennett et al. 1992).

HOST RANGE

Over 130 species of *Haemoproteus* have been reported from 72 families of birds, depending on authority (Peirce 2005; Valkiūnas 2005). Diversity in terms of number of distinct morphological forms and species is highest among the Coraciiformes (kingfishers), Piciformes (woodpeckers), and Galliformes, but the highest number of species occurs within the Passeriformes (perching birds) (Bennett 1993). Of interest is the wide disparity in occurrence of haemoproteid infections among the avian orders (Bennett 1993; Valkiūnas 2005). *Haemoproteus* has not been reported in many of the more primitive orders of birds, but is very common among the Passeriformes (Table 2.1). Some of these differences are clearly related to vector distribution

Table 2.1. Host distribution of avian haemoproteids by avian order.

Avian order	Host species	Number examined	Number infected	Percent infected
Sphenisciformes	16	16	0	0
Gaviiformes	4	3	0	0
Podicipediformes	21	7	0	0
Procellariiformes	100	30	0	0
Pelecaniformes	57	44	0	0
Tinamiformes	47	12	0	0
Apterygiformes	3	1	0	0
Struthioniformes	8	4	0	0
Ciconiiformes	124	89	40	45
Falconiformes	296	168	83	49
Strigiformes	162	66	49	74
Anseriformes	154	113	56	50
Galliformes	270	133	74	56
Gruiformes	203	87	47	54
Charadriiformes	339	154	36	23
Columbiformes	323	135	87	64
Psittaciformes	344	143	43	30
Cuculiformes	153	84	40	48
Caprimulgiformes	106	51	8	16
Apodiformes	414	75	20	27
Piciformes	402	201	60	30
Coliiformes	6	3	0	0
Coraciiformes	202	118	13	11
Trogoniformes	39	20	7	35
Passeriformes	5,211	2,409	2,047	85

Note: Data are summarized from Table 1 in Bennett (1993) and represent number of reported host species for each avian order that are infected with one or more species of *Haemoproteus*.

and abundance, with correspondingly low prevalence in seabirds and shorebirds that have limited exposure to hippoboscid or ceratopogonid flies (Mendes et al. 2005), while others may be related to differences in host resistance and immune competence (Ricklefs 1992; Sol et al. 2003).

ETIOLOGY

Members of this genus are classified as members of the phylum Apicomplexa, class Aconoidasida, order Haemosporida, family Plasmodiidae, and are defined primarily by their intraerthrocytic development, production of prominent golden-brown or black pigment granules from digestion of host hemoglobin, and absence of asexual reproduction in the circulating blood cells (Peirce 2000). Virtually all species in this genus are distinguished by morphology of the circulating gametocytes, their presumed host specificity, and by distinctive changes in host erythrocyte morphology (Figures 2.1a, b, and 2.2). Five different morphological types of gametocytes are recognized that differ in shape (round or elongated) and how far they reach around the erythrocyte nucleus (Figure 2.2).

Recent phylogenetic analyses based on mitochondrial gene sequences have placed *Haemoproteus* as a polyphyletic group within the same clade as *Plasmodium* (Perkins and Schall 2002). More recent analyses based on four genes find that the avian haemoproteids fall into two clades that are sister to *Plasmodium*: a basal group of columbiform parasites that uses hippoboscid flies as vectors and a second distinct group that is transmitted by ceratopogonid flies (Martinsen et al. 2008). These new analyses support the proposal by Bennett et al. (1965) to subdivide the genus, keeping columbiform parasites transmitted by hippoboscid flies in the genus *Haemoproteus* and moving the bulk of species that are likely transmitted by ceratopogonid flies into the genus *Parahaemoproteus* (Martinsen et al. 2008). While this distinction is currently made at the level of subgenus (Valkiūnas 2005), these recent phylogenetic studies suggest that the proposal by Bennett et al. (1965) should be revived.

The most recent taxonomic revisions of this genus are by Peirce (2005) and Valkiūnas (2005). Peirce

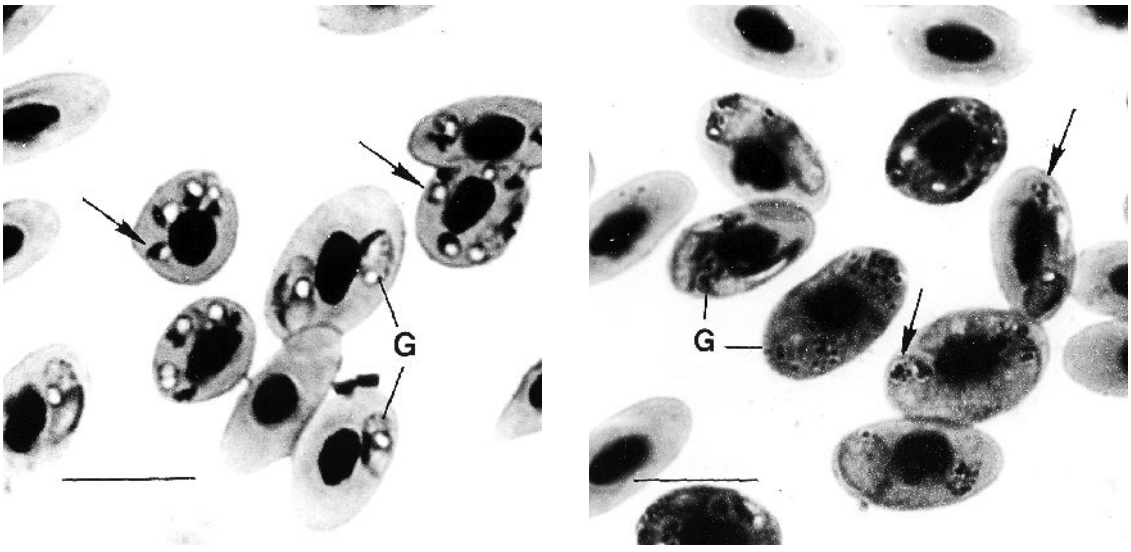

Figure 2.1. Gametocytes of *Haemoproteus meleagridis* in erythrocytes of an experimentally infected domestic turkey. (a) After release from preerythrocytic meronts, merozoites (arrows) invade erythrocytes and develop into mature gametocytes. Intraerythrocytic merozoites have a large vacuole and small nucleus. As merozoites transform into young gametocytes (G), they become elongated and sausage-shaped, eventually encircling the erythrocyte nucleus. As many as seven gametocytes may be found within individual erythrocytes in intense infections. (b) Gametocytes (G) reach maturity within 7 days after invading erythrocytes. Pigment granules (arrows) become visible only during later stages of development. Giemsa stain, bar = 10 μm. Reproduced from Atkinson (1991a), with permission of the *Journal of Vector Ecology*.

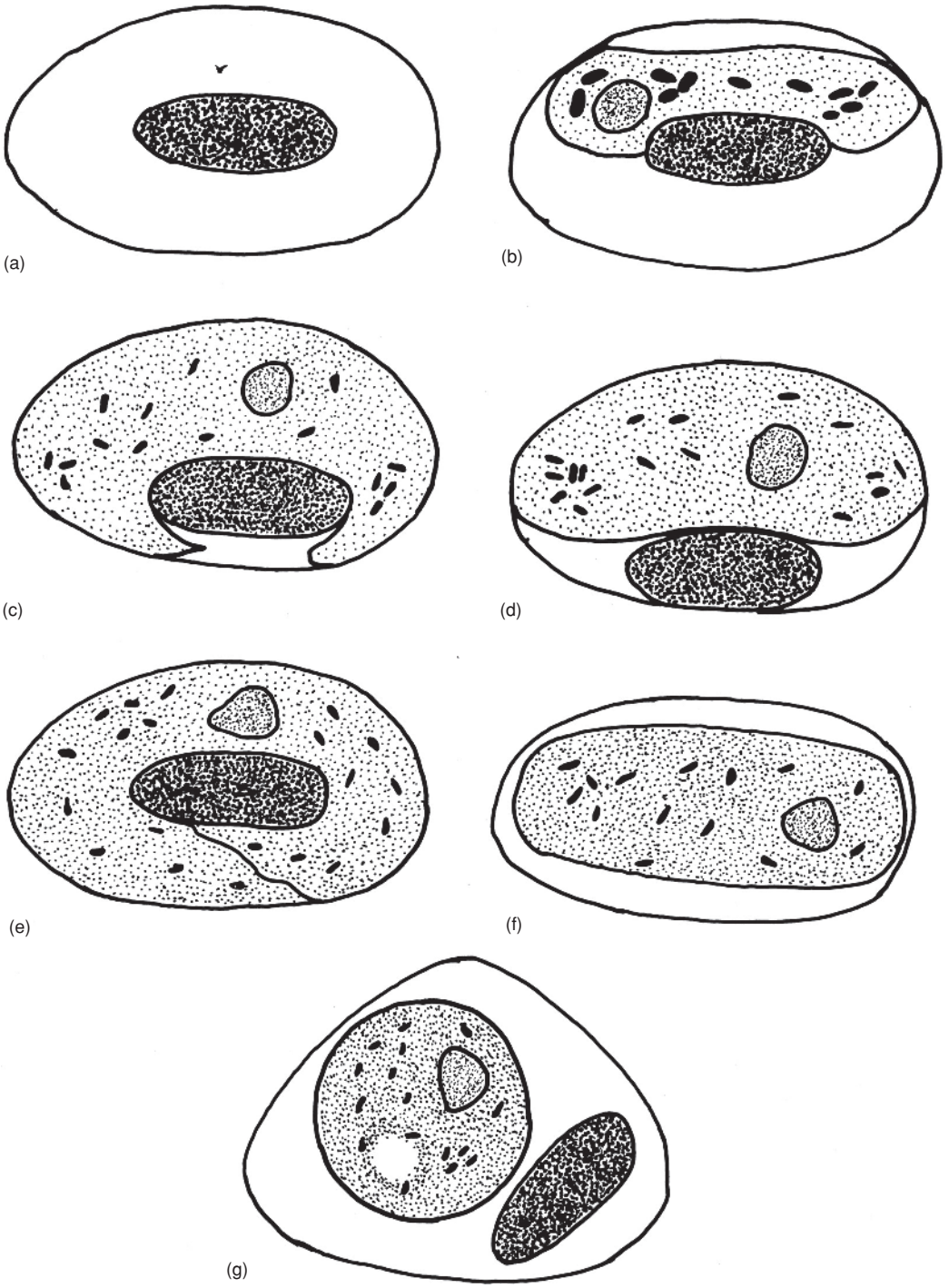

Figure 2.2. Five basic morphological forms of the mature gametocytes of avian species of *Haemoproteus*: (a) normal erythrocyte, (b) microhalteridial gametocyte, (c, d) halteridial gametocyte, (e) circumnuclear gametocyte, (f) rhabdosomal gametocyte, and (g) discosomal gametocyte. Reproduced from Bennett et al. (1988), with permission of the *Journal of Natural History* and Taylor & Francis Ltd. (http://www.informaworld.com).

(2005) lists 147 species in 72 avian families, while Valkiūnas synonymizes some species based on host range and lists 132 valid species. Early efforts by Gorden Bennett and coworkers to make some taxonomic sense out of the bewildering diversity of avian haemoproteids led to separation of morphologically similar forms by host family, based on limited experimental evidence indicating that species are specific to family (Bennett and Peirce 1988). As is the case with closely related parasites in the genus *Leucocytozoon* (Chapter 4), much of this evidence was based on small sample sizes and only a few attempts to actually infect members of different host families and orders (Valkiūnas 2005). Problems with this taxonomy have been summarized by Valkiūnas (2005), and he argues effectively that available evidence only supports specificity to level of host order.

Our understanding of the relationships between traditional morphological species and parasite lineages defined by mitochondrial and nuclear gene sequences is rapidly evolving. Recent studies of diversity of mitochondrial and nuclear genes of avian haemoproteids suggest that the true number of species may be several orders of magnitude higher with multiple parasite lineages that can coexist within the same host and with host ranges that extend well beyond single avian families (Bensch et al. 2000, 2004; Ricklefs and Fallon 2002). As a result, many traditional species defined by morphological characteristics and host family may be composed of multiple cryptic species, while many others that are defined only by occurrence in different host families will need to be synonymized. It is likely that major strides in our understanding of the taxonomy, host specificity, and evolutionary relationships among these parasites and closely related species in the genus *Plasmodium* will occur in future years as molecular data are reconciled with life history characteristics of these organisms.

EPIZOOTIOLOGY

The complex life cycle of *Haemoproteus* involves both sexual (gametogenesis and fertilization) and asexual (sporogony) reproduction in the vector and asexual reproduction (merogony) in the avian host. Proven vectors of avian haemoproteids include both ceratopogonid flies in the genus *Culicoides* and ectoparasitic hippoboscid flies (Table 2.2). The sexual cycle begins when a blood meal containing mature sexual stages of the parasite, female macrogametocytes and male microgametocytes, is taken from an infected host. The sexual stages undergo gametogenesis and fertilization in the midgut of the vector and produce a motile zygote called the ookinete. Ookinetes subsequently penetrate the midgut wall and develop under the midgut basal lamina as spherical oocysts during the asexual sporogonic cycle.

Development of the parasite in both vectors is similar, but size of oocysts, number of sporozoites produced, and duration of sporogony differs. In ceratopogonid flies, oocysts measure approximately 10 μm in diameter, while in hippoboscid flies oocysts are considerably larger and reach diameters of approximately 40 μm (Adie 1924; Fallis and Bennett 1960; Atkinson 1991b). Sporogony typically takes 4–6 days in ceratopogonid flies, eventually producing fewer than 100 sporozoites that bud from a single sporoblast. Sporogony in hippoboscid flies typically takes up to 10 days, eventually producing thousands of sporozoites that bud from multiple sporoblasts within the oocysts (Adie 1915, 1924). Oocysts subsequently rupture, releasing sporozoites into the haemocoel of the insect. These invade the salivary glands and pass through the salivary ducts during the next blood meal.

The factors that affect the ability of particular species of *Haemoproteus* to develop in a particular species of arthropod vector are poorly understood. It is clear that individual species of avian *Haemoproteus* can be transmitted by a number of different hippoboscid or ceratopogonid vectors (Table 2.2), but successful development as measured by ability to complete sporogony and produce sporozoites that can reach the salivary glands varies in each species of *Culicoides* (Atkinson 1991a; Valkiūnas et al. 2002). It is not known whether blocks in development occur in the midgut, during passage through the peritrophic membrane that surrounds the blood meal during digestion, or within the midgut epithelium.

It has never been demonstrated by experimental methods that haemoproteids transmitted by hippoboscid flies can also be transmitted by ceratopogonid flies, although complete development of *Haemoproteus lophortyx* from Northern Bobwhites in *Culicoides bottimeri*, *Stilbometopa impressa*, and *Lynchia hirsuta* suggests that this is possible (O'Roke 1930; Tarshis 1955; Mullens et al. 2006). However, the original experimental work on hippoboscid transmission of *H. lophortyx* (O'Roke 1930; Tarshis 1955) was done in facilities that were not adequately screened to prevent entry by ceratopogonid flies (Valkiūnas 2005; Table 2.2). Among other species of *Haemoproteus*, the rare occurrence of hippoboscid flies on Mourning Doves (*Zenaida macroura*) and high prevalence of *Haemoproteus sacharovi* strongly suggest that ceratopogonid flies may be involved in transmission of this parasite, but this possibility has not been investigated (Bennett and Peirce 1990). Given the results of recent phylogenetic studies (Martinsen et al. 2008), experimental tests of vector specificity of these two groups of haemoproteids should be pursued.

Table 2.2. Known species of hippoboscid (*Lynchia, Microlynchia, Ornithomyia, Pseudolynchia, Stilbometopa*) and ceratopogonid (*Culicoides*) flies that can support complete asexual sporogonic development of *Haemoproteus*.

Species	Host order	Vector	Authors
Haemoproteus nettionis	Anseriformes	*Culicoides downesi*	Fallis and Wood (1957)
Haemoproteus columbae	Columbiformes	*Pseudolynchia canariensis*	Sergent and Sergent (1906)
		Pseudolynchia brunnea	Aragão (1908b)
		Microlynchia pusilla	Aragão (1916)
Haemoproteus sacharovi[*]	Columbiformes	*Pseudolynchia canariensis*	Huff (1932)
Haemoproteus maccallumi	Columbiformes	*Pseudolynchia canariensis*	Huff (1932)
Haemoproteus turtur[†]	Columbiformes	*Pseudolynchia canariensis*	Rashdan (1998)
Haemoproteus palumbis	Columbiformes	*Ornithomyia aviculria*	Baker (1963, 1966)
Haemoproteus lophortyx[‡]	Galliformes	*Stilbometopa impressa*	O'Roke (1930)
		Lynchia hirsuta	Tarshis (1955)
		Culicoides bottimeri	Mullens et al. (2006)
Haemoproteus mansoni	Galliformes	*Culicoides sphagnumensis*	Fallis and Bennett (1960)
Haemoproteus meleagridis[§]	Galliformes	*Culicoides edeni*	Atkinson et al. (1983)
		Culicoides hinmani	Atkinson et al. (1983)
		Culicoides arboricola	Atkinson et al. (1983)
		Culicoides haematopotus	Atkinson (1988)
		Culicoides knowltoni	Atkinson (1988)
Haemoproteus handai	Psittaciformes	*Culicoides nubeculosus*	Miltgen et al. (1981)
Haemoproteus velans	Passeriformes	*Culicoides stilobezziodes*	Khan and Fallis (1971)
		Culicoides sphagnumensis	Khan and Fallis (1971)
Haemoproteus fringillae	Passeriformes	*Culicoides crepuscularis*	Fallis and Bennett (1961)
		Culicoides stilobezziodes	Fallis and Bennett (1961)
		Culicoides sphagnumensis	Fallis and Bennett (1961)
		Culicoides impunctatus	Valkiūnas (1997)
Haemoproteus danilewskii	Passeriformes	*Culicoides crepuscularis*	Bennett and Fallis (1960)
		Culicoides stilobezziodes	Bennett and Fallis (1960)
		Culicoides sphagnumensis	Fallis and Bennett (1961)
		Culicoides edeni	Garvin and Greiner (2003a)
		Culicoides knowltoni	Garvin and Greiner (2003a)
		Culicoides arboricola	Garvin and Greiner (2003a)
Haemoproteus balmorali	Passeriformes	*Culicoides impunctatus*	Valkiūnas et al. (2002)
Haemoproteus dolniki	Passeriformes	*Culicoides impunctatus*	Valkiūnas et al. (2002)
Haemoproteus tartakovskyi	Passeriformes	*Culicoides impunctatus*	Valkiūnas et al. (2002)
Haemoproteus belopolskyi	Passeriformes	*Culicoides impunctatus*	Valkiūnas and Iezhova (2004)
Haemoproteus lanii	Passeriformes	*Culicoides impunctatus*	Valkiūnas and Iezhova (2004)

[*]Circumstantial evidence indicates that one or more species of *Culicoides* may also be involved in natural transmission of *Haemoproteus sacharovi* (Bennett and Peirce 1990).

[†]*Haemoproteus turtur* is recognized as distinct from *Haemoproteus columbae* by Valkiūnas (2005), but considered a synonym of *H. columbae* by Peirce (2005).

[‡]Tarshis (1955) was unable to demonstrate sporozoites of *Haemoproteus lophortyx* in *Stilbometopa impressa* and *Lynchia hirsuta* in spite of repeated attempts to infect them in the laboratory, but did successfully transmit *H. lophortyx* when flies were allowed to bite uninfected birds. Valkiūnas (2005) suggests that experimental cages may not have been impervious to ceratopogonid flies, based on unusually long prepatent periods for experimental infections and use of screened outdoor aviaries. O'Roke (1930), however, describes oocysts and sporozoites in *L. hirsuta* that fed on infected quail. Given the recent finding that *Culicoides bottimeri* is a likely natural vector, experiments with both *S. impressa* and *L. hirsuta* should be repeated.

[§]*Haemoproteus meleagridis* is considered a junior synonym of *Haemoproteus canachites* by Valkiūnas (2005).

Complete life cycles are known for only a handful of avian haemoproteids and we still have only a rudimentary knowledge about the preerythrocytic development of these parasites. *Haemoproteus columbae* from pigeons and doves, *Haemoproteus meleagridis* from Wild Turkeys, and *Haemoproteus danilewskii* from Blue Jays (*Cyanocitta cristata*) have received the most detailed experimental study.

Endogenous development of all three of these species begins when infective sporozoites are inoculated at the site where the vector takes a blood meal. These sporozoites develop within cells of the lymphoid–macrophage system, capillary endothelium, and/or myofibroblasts, undergoing one or more generations of asexual reproduction or merogony before penetrating circulating erythrocytes (Mohammed 1965; Atkinson et al. 1986). Here they develop as gametocytes, becoming infective to vectors within 7–10 days after invading the blood cells.

At least two generations of preerythrocytic merogony occur in skeletal and cardiac muscle of domestic turkeys experimentally infected with *H. meleagridis*. The first begins when infective sporozoites invade capillary endothelial cells and myofibroblasts and develop into thin-walled round or oval meronts measuring 12–20 μm in diameter. These produce long, slender merozoites between 5 and 8 days postinfection that subsequently invade new capillary endothelial cells in skeletal and cardiac muscle and develop as second-generation meronts. Early second-generation meronts are 5–8 μm in diameter and 28 μm in length. These grow rapidly to form large, fusiform, thick-walled megalomeronts measuring up to 500 μm in length (Figure 2.3). Megalomeronts reach maturity at 17 days postinfection and rupture to release small spherical merozoites that invade erythrocytes and develop into gametocytes (Figure 2.1a). Mature gametocytes that completely encircle the host erythrocyte nucleus develop within 7–10 days after red blood cells are invaded (Figure 2.1b). Parasitemias reach their peak intensity in the peripheral circulation at approximately 21 days postinfection and fall rapidly within 7 days to low intensities. A second, smaller peak in parasitemia may occur at approximately 35 days postinfection (Atkinson et al. 1986). The number of generations of preerythrocytic merogony has not been defined for *H. columbae* and *H. danilewskii*, but it is likely that they also undergo two or more cycles of asexual reproduction before invading erythrocytes. In these two species, the parasites invade capillary endothelial cells of the lungs where they undergo preerythrocytic development to form thin-walled, oval or branching meronts that radiate along pulmonary capillaries (Mohammed 1965; Garnham 1966; Garvin et al. 2003a; Valkiūnas 2005). Similar, thin-walled branching meronts have been reported in a variety of other naturally infected avian hosts (Figure 2.4). Thick-walled megalomeronts have been reported in Luzon Bleeding-hearts (*Gallicolumba luzonica*) infected with *H. columbae* (Earle et al. 1993), but their relationship to the thin-walled, branching meronts of *H. columbae* described from Rock Pigeons is unclear and possibly related to presence of a mixed infection with another unidentified parasite (Peirce et al. 2004).

Among haemoproteids transmitted by *Culicoides*, prepatent periods vary from 11 to 12 days for

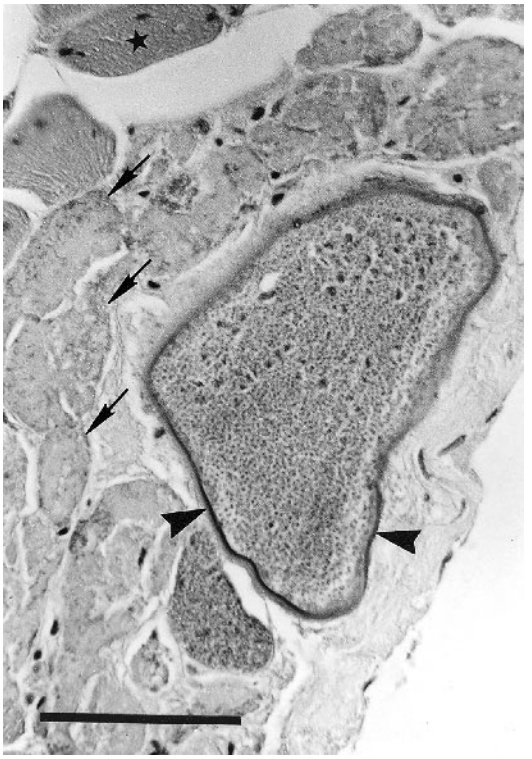

Figure 2.3. Megalomeront of *Haemoproteus meleagridis* from the pectoral muscle of a naturally infected Wild Turkey (*Meleagris gallopavo*). The megalomeront is surrounded by a thick, hyaline wall (arrowheads) and is packed with spherical merozoites. Muscle fibers surrounding the megalomeront are swollen, pale, and hyaline and contain scattered basophilic granules (arrows). Note adjacent normal tissue (*). Hematoxylin and eosin, bar = 50 μm. Reproduced from Atkinson and Forrester (1987), with permission of the *Journal of Wildlife Diseases*.

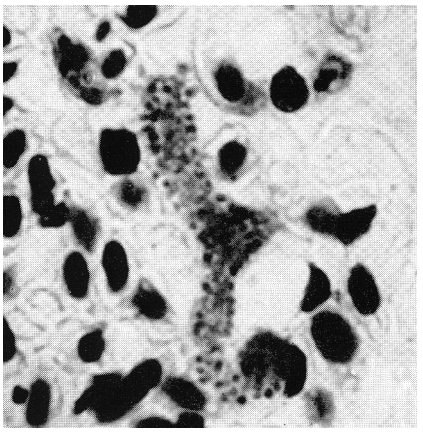

Figure 2.4. Thin-walled, irregularly shaped meront in lung tissue from a White-throated Sparrow (*Zonotrichia albicollis*) infected naturally with *Haemoproteus coatneyi*. Reproduced from Khan and Fallis (1969), with permission of the *Canadian Journal of Zoology*.

Haemoproteus belopolskyi of Blackcaps (*Sylvia atricapilla*) (Valkiūnas and Iezhova 2004), from 11 to 14 days for *Haemoproteus velans* of woodpeckers (Khan and Fallis 1971), 14 days for *Haemoproteus mansoni* of Ruffed Grouse (*Bonasa umbellus*) (Fallis and Bennett 1960), approximately 16 days for *Haemoproteus nettionis* of ducks (Fallis and Wood 1957), 14 days for *H. danilewskii* of Blue Jays (Garvin et al. 2003a), and 17 days for *H. meleagridis* of Wild Turkeys (Atkinson et al. 1986).

Among haemoproteids transmitted by hippoboscid flies, the prepatent period ranges from 17 to 37 days for *H. columbae* of Rock Pigeons and is about 14 days for *Haemoproteus palumbis* of Common Wood-Pigeons (*Columba palumbus*) (Baker 1966). Like the species of *Haemoproteus* that are transmitted by *Culicoides*, merozoites in circulating erythrocytes develop to mature microgametocytes and macrogametocytes that encircle the erythrocyte nucleus within approximately 5–10 days. Gametocyte numbers peak in the peripheral circulation approximately 10–20 days after first appearing in the circulation and then decline in numbers.

Among species of *Haemoproteus* transmitted by ceratopogonid flies, transmission is seasonal and limited to the spring and summer months in more temperate parts of their range (Bennett and Fallis 1960), but can occur throughout the year in subtropical habitats in Florida and most likely other parts of the world where suitable vectors are present year round (Atkinson et al. 1988a). In temperate North America, by contrast, transmission of *H. columbae* by hippoboscid flies is seasonal and closely correlated with changes in vector populations, generally increasing in the fall and winter months and then declining as vector density decreases (Klei and DeGiusti 1975). More limited data from tropical and subtropical parts of the world where populations of hippoboscid flies remain more constant indicate that high rates of transmission and high prevalences of infection can be maintained throughout the year (Ayala et al. 1977; Sol et al. 2000).

The role that host migratory behavior plays in cycles of transmission of avian haemoproteids is significant because of the potential of long distance migrants to disperse parasites both within and between continental landmasses (Laird 1960; Waldenström et al. 2002; Hasselquist et al. 2007). Limited information from the Nearctic and Palearctic indicates that some species of avian haemoproteids are transmitted on the breeding grounds, while others are transmitted in wintering areas in the tropics and subtropics, while others may be transmitted in both locations. This suggests that transmission may be linked in some cases to particular geographic locations or vector–parasite associations (Valkiūnas 1993; Valkiūnas and Iezhova 2001; Waldenström et al. 2002; Garvin et al. 2003b, 2004; Hasselquist et al. 2007; Hellgren et al. 2007b).

Within individual hosts, intensity of infection varies after the initial acute phase and appears to be influenced by the complex interplay of host immunity, seasonal changes in photoperiod, and hormonal changes associated with reproduction. In temperate climates, a seasonal increase in intensity, termed the spring relapse, coincides with the breeding season when populations of blood-sucking insects typically increase and recently fledged susceptible birds are increasing in the population (Atkinson and van Riper 1991; Valkiūnas et al. 2004). Relapse of chronic *Plasmodium* infections can be triggered by corticosterone (Applegate and Beaudoin 1970) and other experimental evidence suggests that increases in photoperiod and subsequent physiological changes in levels of hormones such as melatonin that regulate circadian rhythms may also be important stimuli for initiating relapses among species of *Haemoproteus* (Valkiūnas et al. 2004).

Other factors affecting intensity include stress-mediated changes in the immune system that are associated with reproductive effort (Siikamäki et al. 1997), food availability (Appleby et al. 1999), concomitant infection with other parasites (Cox 1987), and exposure to predators (Navarro et al. 2004).

Attempts to identify broad patterns and relationships in the prevalence of avian haemoproteids have met with variable success because of the diversity of this group of parasites. Much may depend on how prevalence data

are lumped, with positive relationships more evident where other species of hematozoans are included in the analyses. A wide variety of both intrinsic and extrinsic factors have been identified, including host specificity of the parasites (Bennett 1993), immune competency (Ricklefs 1992), host genotype (Bonneaud et al. 2006), host age and sex (Davidar and Morton 1993; Powers et al. 1994; McCurdy et al. 1998), geographic range of host species (Tella et al. 1999), whether or not host species are migratory (Bennett and Fallis 1960; Peirce and Mead 1978; Figuerola and Green 2000; Smith et al. 2004), plumage coloration (Yezerinac and Weatherhead 1995), and host foraging or nesting behavior (Greiner et al. 1975; Garvin and Remsen 1997).

Extrinsic factors such as habitat, geographical region, and season are critically important because they can influence the distribution and abundance of vectors (Weatherhead and Bennett 1991, 1992; Sol et al. 2000; Mendes et al. 2005). Prevalence in the same host species can vary significantly across both large and small landscapes (Atkinson et al. 1988a; Sol et al. 2000; Wood et al. 2007), suggesting that vector distribution and abundance may be the most important determinant of prevalence. However, other factors may cause seasonal changes in parasite prevalence, including winter mortality in infected birds, and new infections associated with emergence of insect vectors and transmission to uninfected juvenile birds.

CLINICAL SIGNS

Clinical signs are usually not evident in low-intensity infections, but can become evident during acute phase infections when erythrocytic parasitemias and numbers of tissue meronts reach high intensities. Domestic turkey poults with experimental infections of *H. meleagridis* are lame in one or both legs and have lower weights and growth rates than do uninfected controls (Atkinson et al. 1988b). Similarly, Northern Bobwhites with natural infections of *H. lophortyx* are reluctant to move, have a ruffled, depressed appearance, and exhibit neurological signs such as loss of balance and difficulty walking (Cardona et al. 2002). Signs of infection in Rock Pigeons include weakness, anemia, and anorexia (Acton and Knowles 1914; Coatney 1933).

Elevation in numbers of circulating lymphocytes, heterophils, basophils, eosinophils, and monocyte numbers has been observed in both natural and experimental infections with *Haemoproteus*, and it is likely that these increases represent a cell-mediated response to both erythrocytic and preerythrocytic stages of the parasite, particularly as the latter mature and rupture to release merozoites that invade erythrocytes (Ots and Hõrak 1998; Garvin et al. 2003a). No significant overall difference in plasma protein concentration, hemoglobin concentration, packed cell volume, or weight was observed between infected and uninfected Blue Jays (Garvin et al. 2003a). Other studies have also failed to report significant anemia in infections with *Haemoproteus*, including *H. meleagridis* in experimentally infected domestic turkeys (Atkinson et al. 1988b) and *Haemoproteus* spp. in Great Tits (*Parus major*) (Ots and Hõrak 1998). By contrast, O'Roke (1930) and Cardona et al. (2002) detected severe anemia in California Quail (*Callipepla californica*) and captive Northern Bobwhites with natural infections of *H. lophortyx*. Severe regenerative anemia with marked polychromasia has also been reported in Snowy Owls (*Bubo scandiacus*) infected with *Haemoproteus noctuae* (Evans and Otter 1998) and in Snowy Owls, Tawny Owls (*Strix aluco*), and Great Horned Owls (*Bubo virginianus*) infected with *Haemoproteus syrnii* (Mutlow and Forbes 1999). Mechanisms responsible for development of anemia in these host species are not known, although there may be a fine balance between removal of parasitized erythrocytes by the spleen and their replacement with immature red blood cells (Atkinson et al. 1988b). When an infected host lacks the physiological resources to replace infected blood cells because of stress associated with reproduction or limited food resources, anemia may result.

PATHOGENESIS AND PATHOLOGY

Virtually nothing is known about the pathogenesis of haemoproteid infections because so little is known about their development within natural and experimental hosts. Few host responses have been associated with development of thin-walled branching meronts that frequently occur in lung tissue (Mohammed 1965; Baker 1966; Garnham 1966) (Table 2.3). In one of the most detailed studies to date, no host responses were associated with preerythrocytic meronts at day 31 postinfection in Blue Jays infected experimentally with *H. danilewskii*. However by day 57 postinfection, juvenile jays had lesions in liver, spleen, and lung tissue. These included periportal and random individual cell necrosis in liver and lymphocytic infiltrates and epithelial hyperplasia around tertiary bronchi in lung tissue. Histological changes in splenic tissue included hyperplasia of white pulp arteriolar endothelium, random necrosis of lymphocytes, and increases in the number of macrophages, plasma cells, and Mott cells (Garvin et al. 2003a). The authors suggested that the lesions developed only after meronts matured and ruptured.

Severe myositis has been reported in association with thick-walled megalomeronts in a variety of avian species (Table 2.3). These lesions are associated with intact and ruptured megalomeronts and are grossly visible as white flecks or dark hemorrhagic streaks

Table 2.3. Preerythrocytic meronts and host responses reported from wild (W), domestic (D), captive (C), or experimentally infected (E) avian hosts.

Host species	Host order	Parasite	Status	Tissue	Pathology	Citations
Thin-walled oval or branching meronts						
Wood Duck (*Aix sponsa*)	Anseriformes	*Haemoproteus nettionis*	E	Lungs, heart, spleen	No	Sibley and Werner (1984)
Black Crowned-Crane (*Balearica pavonina*)	Gruiformes	*Haemoproteus balearicae*	W	Lung	No	Peirce (1973)
Rock Pigeon (*Columba livia*)	Columbiformes	*Haemoproteus columbae*	W, E, D	Lungs, rarely liver and spleen	None reported or tissue displacement, blockage of vessels	Aragão (1908b), Mohammed (1965), and Peirce et al. (2004)
Common Wood-Pigeon (*Columba palumbus*)	Columbiformes	*Haemoproteus palumbis*	W	Lungs, heart	No	Baker (1966)
Mourning Dove (*Zenaida macroura*)	Columbiformes	*Haemoproteus sacharovi*	W	Lung	No	Greiner (1971)
Mourning Dove (*Zenaida macroura*)	Columbiformes	*Haemoproteus maccallumi*	W	Lung	No	Greiner (1971)
Blue Jay (*Cyanocitta cristata*)	Passeriformes	*Haemoproteus danilewskii*	E	Liver, spleen, lung	Minor inflammation	Garvin et al. (2003a)
House Sparrow (*Passer domesticus*)	Passeriformes	*Haemoproteus passeris*	W	Lungs, liver	No	Peirce (1976)
Noisy Miner (*Manorina melanocephala*)	Passeriformes	*Haemoproteus ptilotis**	W	Heart and spleen	Tissue displacement, inflammation	Peirce et al. (2004)
Noisy Friarbird (*Philemon corniculatus*)	Passeriformes	*Haemoproteus ptilotis**	W	Liver	No	Peirce et al. (2004)
European Robin (*Erithacus rubecula*)	Passeriformes	*Haemoproteus attenuatus*	W	Lungs, spleen	No	Valkiūnas (2005)
White-throated Sparrow (*Zonotrichia albicollis*)	Passeriformes	*Haemoproteus coatneyi*	W	Lungs, heart, liver, spleen, cecum, kidneys	No	Khan and Fallis (1969)

Thick-walled meglomeronts						
Lesser Flamingo (*Phoenicopterus minor*)	Phoenicopteriformes	*Haemoproteus* sp.†	C	Liver	Hepatic necrosis, hemorrhage, inflammation	Ferrell et al. (2007)
Muscovy Duck (*Cairina moschata*)	Anseriformes	Undetermined*	D	Heart, lungs, liver, kidneys, spleen	Inflammation	Commichau and Jonas (1977) and Kŭcera et al. (1982)
Northern Bobwhite (*Colinus virginianus*)	Galliformes	*Haemoproteus lophortyx*	C	Skeletal muscle	Myopathy	Cardona et al. (2002)
Domestic Turkey, Wild Turkey (*Meleagris gallopavo*)	Galliformes	*Haemoproteus meleagridis*	W, E	Cardiac and skeletal muscle	Myopathy	Atkinson and Forrester (1987) and Atkinson et al. (1988b)
Domestic chicken, Red Jungle Fowl (*Gallus gallus*)	Galliformes	*Arthrocystis galli**	D	Skeletal and cardiac muscle	Myopathy	Levine et al. (1970) and Opitz et al. (1982)
Luzon Bleeding-heart (*Gallicolumba luzonica*)	Columbiformes	*Haemoproteus columbae*	C	Cardiac and skeletal muscle, gizzard, proventriculus	Myopathy	Earle et al. (1993)
Mourning Dove (*Zenaida macroura*)	Columbiformes	*Haemoproteus sacharovi*	W	Gizzard	No	Farmer (1965)
Blossom-headed Parakeet (*Psittacula roseata*)	Psittaciformes	*Haemoproteus handai*	C	Cardiac and skeletal muscle	Myopathy	Miltgen et al. (1981)
Monk Parakeet (*Myiopsitta monachus*)	Psittaciformes	Undetermined*	C	Skeletal muscle	Myopathy	Borst and Zwart (1972)
Parakeet (species not reported)	Psittaciformes	Undetermined*	C	Heart, gizzard	Hemorrhage	Fowler and Forbes (1972) and Walker and Garnham (1972)

(*continues*)

Table 2.3. (*Continued*)

Host species	Host order	Parasite	Status	Tissue	Pathology	Citations
Snowy Owl (*Bubo scandiacus*)	Strigiformes	*Haemoproteus syrnii*	W	Skeletal muscle	No	Mutlow and Forbes (1999)
Israeli House Sparrow (*Passer domesticus biblicus*)	Passeriformes	*Haemoproteus passeris*	W	Liver, lungs, kidney	Inflammation	Garnham (1966) and Paperna and Gil (2003)
Java Sparrow (*Padda oryzivora*)	Passeriformes	Undetermined*	W	Kidney	No	Garnham (1966)
Sacred Kingfisher (*Todiramphus sanctus*)	Passeriformes	*Haemoproteus halcyonis*	W	Skeletal muscle	No	Peirce et al. (2004)
Pied Currawong (*Strepera graculina*)	Passeriformes	Undetermined*	W	Heart, skeletal muscle, gizzard	Myopathy	Lederer et al. (2002)
Green Jay (*Cyanocorax yncas*)	Passeriformes	*Haemoproteus* sp.†	C	Liver	Hepatic necrosis, hemorrhage, inflammation	Ferrell et al. (2007)
Montezuma Oropendola (*Gymnostinops montezuma*)	Passeriformes	*Haemoproteus* sp.†	C	Liver	Hepatic necrosis, hemorrhage, inflammation	Ferrell et al. (2007)

Note: Two primary types of preerythrocytic meronts have been reported: thin-walled, oval or branching forms that are associated with limited host reaction; and thick-walled, round or fusiform forms that occur in skeletal, gizzard, and cardiac muscle, as well as liver, spleen, and lung tissue. The most definitive associations are in birds with experimental infections with *Haemoproteus nettionis*, *Haemoproteus columbae*, *Haemoproteus meleagridis*, and *Haemoproteus danilewskii*. Remaining examples should be viewed with caution since hosts may have been infected with more than one parasite and often did not have circulating gametocytes. Table includes hosts infected with megalomeronts of undetermined or questionable taxonomic status that are suspected to belong to species of *Haemoproteus*.

*No parasitemia observed, possibly *Leucocytozoon* or other undetermined protozoan.

†Identity determined by PCR amplification and sequencing of parasite cytochrome *b* gene.

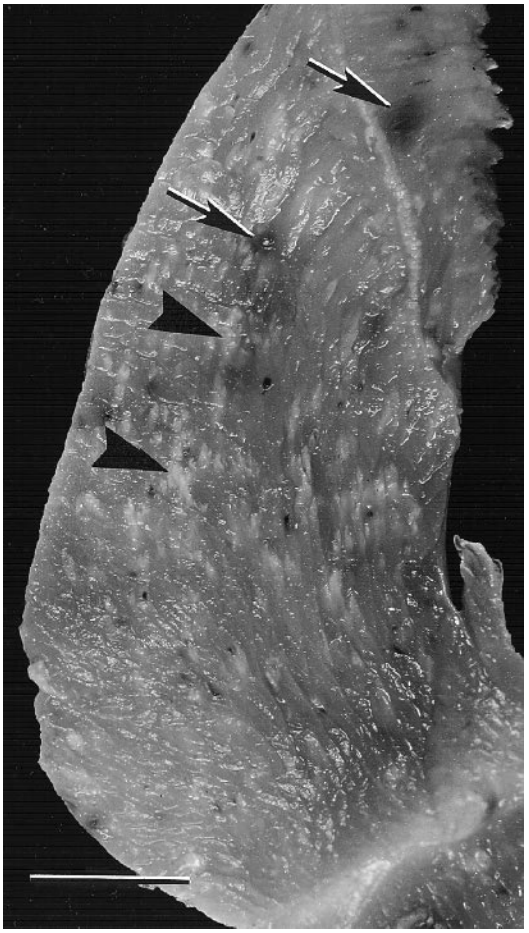

Figure 2.5. Formalin-fixed pectoral muscle from a domestic turkey with an experimental infection with *Haemoproteus meleagridis*. Note the scattered white streaks (arrowheads) and darkened hemorrhagic areas (arrows) that correspond to megalomeronts in histological sections. Hematoxylin and eosin, bar = 0.5 cm. Reproduced from Atkinson et al. (1988b), with permission of the *Journal of Parasitology*.

in skeletal and cardiac muscle. The lesions superficially resemble those from infections with *Sarcocystis* (Figure 2.5). Microscopically, megalomeronts are surrounded by mixed inflammatory infiltrates composed of macrophages, heterophils, giant cells, and red blood cells, and adjacent muscle fibers are often necrotic and calcified (Miltgen et al. 1981; Atkinson et al. 1988b; Cardona et al. 2002) (Figures 2.6 and 2.7). Other lesions include extensive deposition of parasite pigment in tissue macrophages of the liver and spleen and enlargement of these organs (Atkinson et al. 1986, 1988b; Atkinson and Forrester 1987).

Megalomeronts with associated muscle pathology have been reported in a variety of other naturally infected avian hosts, but their role in life cycles of specific species of *Haemoproteus* is difficult to determine without experimental studies (Peirce et al. 2004; Table 2.3).

DIAGNOSIS

The gold standard for diagnosis of *Haemoproteus* is a Giemsa-stained thin blood smear where it is possible to demonstrate the presence of erythrocytic gametocytes with prominent golden-brown or black pigment granules and absence of erythrocytic meronts that are diagnostic for *Plasmodium* spp. Individual species are traditionally defined by morphology of intraerythrocytic gametocytes (Figure 2.2) and host specificity, but this will likely undergo extensive revision in future years. Molecular methods are beginning to be applied to differentiation of genera and identification of unique parasite lineages. Their high sensitivity make them valuable for identifying birds with very low intensity infections, but these methods have not been refined to the point where they can be used to distinguish individual species. Recent studies, though, suggest that this may eventually be feasible (Hellgren et al. 2007a; Valkiūnas et al. 2007).

Species of *Haemoproteus* may be difficult to distinguish from avian species of *Plasmodium*, particularly in chronic infections where number of circulating gametocytes is low and where it may be difficult to determine whether the intracellular meronts characteristic of *Plasmodium* are present or absent. Several recent sets of primers designed to amplify portions of parasite mitochondrial genome can distinguish *Haemoproteus* and *Plasmodium* from *Leucocytozoon* (Hellgren et al. 2004) or all three genera from each other following restriction digests of polymerase chain reaction (PCR) products (Beadell and Fleischer 2005). However, sequencing of PCR products is necessary for identifying individual parasite lineages and determining phylogenetic relationships.

The morphology of tissue stages is difficult to use alone for making accurate diagnosis of infection with *Haemoproteus*. The thin-walled oval or branching meronts that are characteristic of some species of columbiform haemoproteids are similar in morphology to tissue stages of both *Leucocytozoon* and *Plasmodium*. Megalomeronts of *Haemoproteus* may be difficult to distinguish from those of *Leucocytozoon*. A variety of megalomeronts have been reported as aberrant *Leucocytozoon* infections (Levine et al. 1970; Borst and Zwart 1972; Fowler and Forbes 1972; Walker and

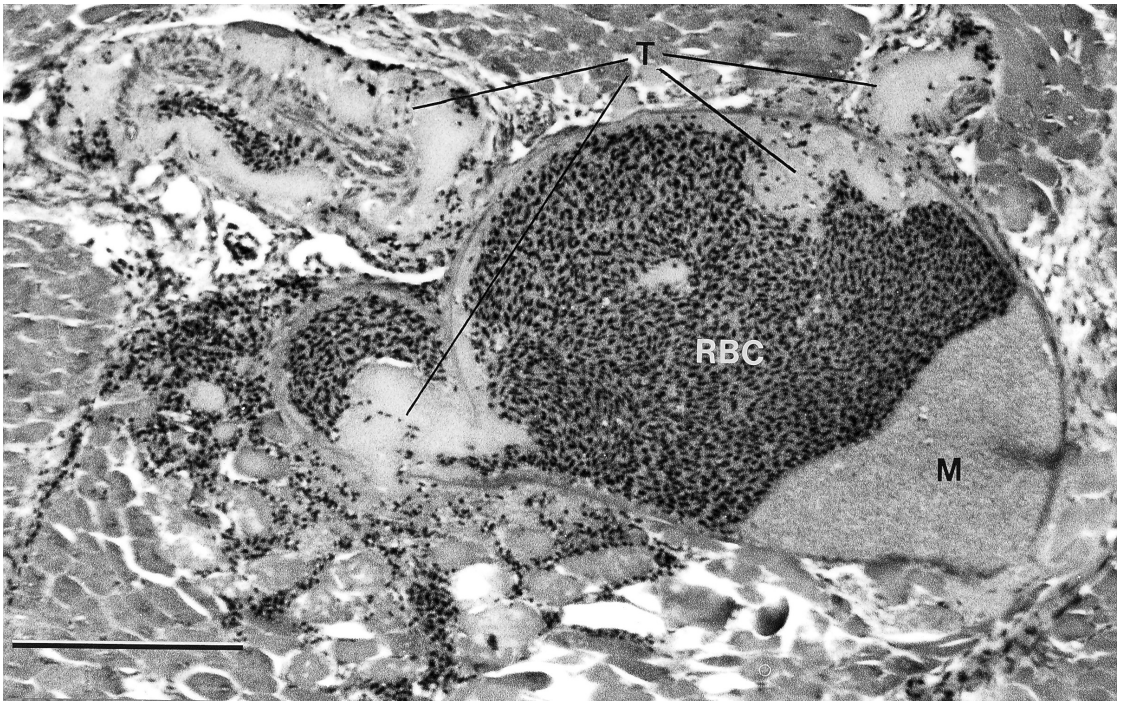

Figure 2.6. Ruptured megalomeront from pectoral muscle of a domestic turkey with an experimental infection with *Haemoproteus meleagridis*. Hemorrhagic megalomeront (M) is surrounded and partially filled by red blood cells (RBCs). Thrombi (T) with embedded RBCs are adjacent to or within the megalomeront. Hematoxylin and eosin, bar = 100 μm. Reproduced from Atkinson et al. (1988b), with permission of the *Journal of Parasitology*.

Garnham 1972; Hartley et al. 1981; Simpson 1991; Pennycott et al. 2006) or possible *Besnoitia* infections (Bennett et al. 1993; Peirce et al. 2004), but erythrocytic parasites were absent and it is possible that some of these reports may prove to be tissue stages of *Haemoproteus*. The difficulties in making diagnoses from wild birds that may be infected with multiple species of haemosporidians have been discussed by Lederer et al. (2002) who pointed out that accurate association between megalomeronts and infection with *Haemoproteus* or *Leucocytozoon* in wild birds requires experimental studies. The recent use of molecular methods for diagnosis may help resolve some of these problems. For example, hepatic megalomeronts in three species of captive birds in a zoo collection in Texas were recently shown to be associated with infection with an undetermined species of *Haemoproteus* by PCR amplification of a portion of the parasite mitochondrial cytochrome *b* gene (Ferrell et al. 2007).

Haemoproteus appears to be antigenically distinct from *Plasmodium* and crude antigen extracts have been used to develop an ELISA test for *H. columbae* in Rock Pigeons (Graczyk et al. 1994). The specificity and sensitivity of this serological test with other avian haemoproteids are not known, but they may prove useful for making genus level diagnoses in birds with low-intensity infections.

IMMUNITY

Virtually nothing is known about immune mechanisms in haemoproteid infections. Spontaneous recovery from infections with *H. columbae* has been reported in Rock Pigeons, with no immunity conferred to second infection (Sergent and Béquet 1914; Ahmed and Mohammed 1978). In most cases birds probably remain infected for long periods of time and have spontaneous relapses that may decrease in frequency, eventually leading to recovery (Coatney 1933; Ahmed and Mohammed 1978). Experimental evidence for this is very limited, however, and restricted to *H. columbae* of Rock Pigeons. Limited experimental data indicate that birds with chronic infections have concomitant immunity where a persistent chronic infection stimulates immunity to reinfection with homologous parasites of the same species (Coatney 1933; Ahmed and Mohammed

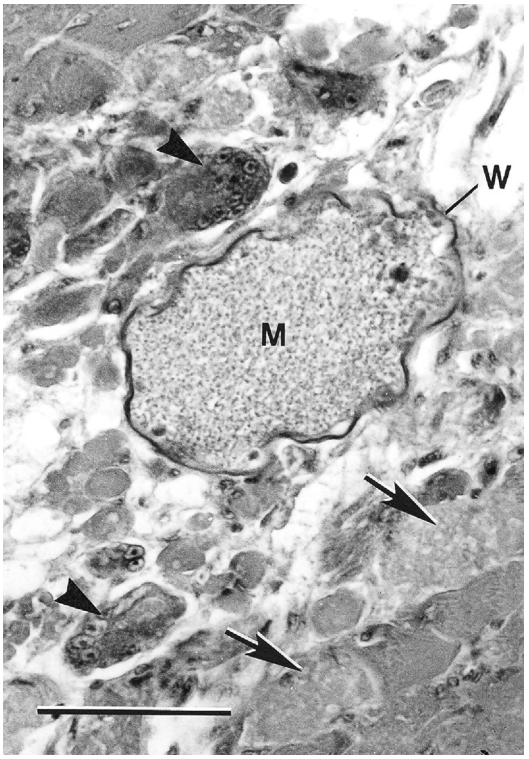

Figure 2.7. Intact megalomeront from pectoral muscle of a domestic turkey with an experimental infection with *Haemoproteus meleagridis*. Megalomeront (M) is surrounded by giant cells (arrowheads) and hyaline and necrotic muscle fibers (arrows). Note thick hyaline wall (W) surrounding the megalomeront. Hematoxylin and eosin, bar = 50 μm. Reproduced from Atkinson et al. (1988b), with permission of the *Journal of Parasitology*.

1978). The relapses associated with chronic infections most likely originate from persistent tissue stages, but this has not been proven by experimental studies.

PUBLIC HEALTH CONCERNS

Infected birds pose no health hazards to humans.

DOMESTICATED ANIMAL HEALTH CONCERNS

Haemoproteus meleagridis of Wild Turkeys is a potential threat to domestic turkey production, but in practice this has never materialized—possibly because of separation of most commercial poultry facilities from habitats where Wild Turkeys range.

There are multiple reports of pathogenic infections of *Haemoproteus* in pigeons and doves. These are usually associated with high parasitemias (Coatney 1933) and the occurrence of megalomeronts (Farmer 1965; Earle et al. 1993), but most individuals appear to be able to tolerate very high parasitemias with no clinical signs of infection.

Major outbreaks of infection with *H. lophortyx* have been reported in Northern Bobwhite raised in California where the natural reservoir host is California Quail. Outbreaks occur during warm weather when ceratopogonid populations increase (Cardona et al. 2002). Similarly, there have been a substantial number of reports of lethal *Leucocytozoon*-like infections affecting captive birds, particularly parakeets, that may actually be caused by species of *Haemoproteus* (Fowler and Forbes 1972; Smith 1972; Walker and Garnham 1972; Simpson 1991; Pennycott et al. 2006; Ferrell et al. 2007). In all these instances, captive birds were introduced to areas outside of their natural range.

WILDLIFE POPULATION IMPACTS

The effects of individual *Haemoproteus* infections are difficult to discern in wild hosts. The vast majority of studies are correlational and the avian hosts under investigation are frequently infected with other hematozoan parasites, including *Leucocytozoon*, *Plasmodium*, and *Trypanosoma*. In a thorough review of over 5,000 papers on avian blood parasites, Bennett et al. (1993) found that only about 4% reported mortality or pathogenicity in birds, with most dealing with domestic birds or birds in zoological collections. Mortality associated with *Haemoproteus* and other blood parasites in wild birds probably occurs more frequently than reported because sick individuals may be difficult to find for sampling or recover from the wild for necropsy. Epizootics are often hard to document for small passerines in areas where carcasses are rapidly scavenged (Bennett et al. 1993).

Since the life cycle of *Haemoproteus* requires a vector, experimental manipulations of naturally acquired infections in the wild are difficult. One approach that has been successful is use of a single subcutaneous dose of primaquine to control *H. majoris* and *Leucocytozoon majoris* in naturally infected Eurasian Blue Tits (*Cyanistes caeruleus*) (Merino et al. 2000). The treated group had higher fledging success and lower nestling mortality, but the relative contributions of *Haemoproteus* and *Leucocytozoon* to decreased fledging success were not determined.

Some studies have reported reduced survival in birds infected with *Haemoproteus* (Nordling et al. 1998;

Dawson and Bortolotti 2000; Hõrak et al 2001; Sol et al. 2003) and negative effects on indices of immunity, condition, and reproductive success of their hosts (Allander and Bennett 1995; Ots and Hõrak 1998; Merino et al. 2000; Sanz et al. 2001). While some studies suggest that these changes may be reflected in plumage coloration (Hõrak et al. 2001), others have found limited association (Kirkpatrick et al. 1991). Effects of infection with *Haemoproteus* can also have indirect effects on host reproduction. Female Eurasian Kestrels (*Falco tinnunculus*) with *Haemoproteus*-infected mates laid smaller and later clutches than did females with unparasitized males (Korpimäki et al. 1995). Among American Kestrels (*Falco sparverius*) infected with *Haemoproteus*, pairs with lower intensity infections fledged more young than birds with higher intensities (Apanius 1991).

There is growing evidence for a trade-off between reproductive effort and resistance to parasites that is thought to arise when limited resources must be partitioned between reproductive effort and disease resistance (Chapter 1). Parasite intensity (as measured by numbers of circulating gametocytes) increases with the degree of effort expended in reproduction (Norris et al. 1994; Ots and Horak 1996; Allander 1997; Siikamäki et al. 1997; Nordling et al. 1998) and may decrease when food resources are abundant (Wiehn and Korpimaki 1998).

From other studies of the subclinical impacts of *Haemoproteus* infections on wild birds, results have often been conflicting and dependent on the particular host–parasite association under investigation, whether or not hosts had concurrent infections with other hematozoan parasites, whether stage of infection was acute or chronic, and age of the hosts. A number of studies have been unable to establish a relationship between infection with *Haemoproteus* and survivorship, mating success, reproductive success, host condition, or clinical chemistry (Bennett et al. 1988; Weatherhead and Bennett 1992; Davidar and Morton 1993; Powers et al. 1994; Korpimäki et al. 1995; Dale et al. 1996; Hõrak et al. 1998; Dawson and Bortolotti 2000; Schrader et al. 2003), yet others find subtle effects that are either difficult to detect or are equivocal (Dawson and Bortolotti 2000). For example, no association was detected between infection with *Haemoproteus tinnunculi* and return rates of American Kestrels when data from both sexes were combined, but there was a significant negative association between return rates and intensity of infection in females (Dawson and Bortolotti 2000). This suggests that acute or recrudescing infections may have more impact on host survivorship than chronic, low-intensity infections, but that effects may be subtle and easily masked when data for males and females are combined. By contrast, Purple Martins (*Progne subis*) infected with *Haemoproteus prognei* returned to breeding sites earlier than uninfected birds, and infected females had higher numbers of fledged young than uninfected birds (Davidar and Morton 1993). These authors hypothesized that recovery from acute phases of infection of *Haemoproteus* was evidence of immunological superiority in surviving hosts and may actually be a measure of superior fitness.

TREATMENT AND CONTROL

A number of antimalarial compounds are effective for reducing intensity of parasitemia in both wild and domestic birds with infections with *Haemoproteus*. These include atebrine, plasmochin, chloroquine sulfate, primaquine, and mefloquine (Coatney 1935; Evans and Otter 1998; Mutlow and Forbes 1999; Remple 2004) as well as the antitheilerial drug buparvaquone (El-Metenawy 1999). Other antimalarials may be effective including pyrimethamine, pyrimethamine–sulfadoxine combinations, and tetracyclines, but their effectiveness in birds is not wildly established (Mutlow and Forbes 1999).

In captive situations, infections with *Haemoproteus* can be controlled by housing birds in screened, *Culicoides*-proof facilities and dusting birds to reduce or eliminate ectoparasitic hippoboscid flies.

MANAGEMENT IMPLICATIONS

There are currently no broad-scale strategies for prevention or control of infections with *Haemoproteus* in wild birds. While reduction of vector populations will decrease transmission of species of *Haemoproteus*, this approach is currently not feasible for the many species of ceratopogonids that have larval habitats in damp soil and tree cavities (Blanton and Wirth 1979) or for ectoparasitic hippoboscid flies that occur on wild birds. It is likely that some species of *Haemoproteus* may become emerging disease threats in the event of global climate change as the range of hosts and vectors change, bringing previously isolated populations into contact with vectors and parasites to which they had no prior exposure. On a smaller scale, similar circumstances occur when avian species are transported or relocated outside of their normal range. Good examples are the recent epizootics of *H. lophortyx* in Northern Bobwhites that were relocated in California (Cardona et al. 2002), sporadic reports of myopathy from megalomeronts in captive psittacines (Pennycott et al. 2006), and periodic outbreaks in other captive birds and zoos where new exotic hosts are exposed to endemic vectors and parasites (Ferrell et al. 2007).

DISCLAIMER

Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. government.

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3
Avian Malaria

Carter T. Atkinson

INTRODUCTION

Avian malaria is a common mosquito-transmitted disease of wild birds that is caused by protozoan parasites in the genus *Plasmodium*. Infections are caused by a complex of more than 40 species that differ widely in host range, geographic distribution, vectors, and pathogenicity. The avian species of *Plasmodium* share morphological and developmental features with closely related haemosporidian parasites in the genera *Haemoproteus* and *Leucocytozoon* (Chapters 2 and 4), but are distinguished from both by the presence of asexual reproduction (merogony) in circulating erythrocytes.

While there are numerous reports of individual birds with acute, pathogenic infections with *Plasmodium*, reports of epizootics are rare and mostly associated with captive birds in zoological collections and abnormal host–parasite associations following introductions of parasites or mosquito vectors to remote islands. *Plasmodium relictum*, one of the most widely distributed species of avian malaria (Beadell et al. 2006), continues to play an important role as a limiting factor in the current distribution and abundance of native Hawaiian forest birds (Warner 1968; Woodworth et al. 2005; Foster et al. 2007).

SYNONYMS

Avian malaria, haemoproteosis. Many reports in the recent ecological literature lump *Plasmodium* with *Haemoproteus* and refer to both genera as avian malaria, making it difficult to identify which genus is being discussed. Clear differences in life history characteristics of these two genera justify their continued separation (Valkiūnas et al. 2005) even though they are closely related (Martinsen et al. 2008).

HISTORY

The avian species of *Plasmodium* have played a seminal role as models for human malaria since they were first recognized as common intraerythrocytic parasites of wild birds (Danilewsky 1889). The early years of this field have been reviewed in detail by Hewitt (1940), Garnham (1966), and Valkiūnas (2005), and it is clear that most of the major milestones in the field of human malariology were associated in one way or another with avian parasites. Highlights include the first descriptions of the characteristic pathological lesions of malaria in birds by Danilewsky (1889), the discovery of the mosquito transmission of *P. relictum* by Sir Ronald Ross (1898), discovery of the exoerythrocytic merogony of *Plasmodium elongatum* in reticuloendothelial cells in bone marrow and other organs in birds (Raffaele 1934), and the development of the theory of premunition or a resistance to reinfection that is conferred by a chronic malarial infection in avian hosts (Sergent and Sergent 1956).

It was recognized relatively early that both wild and captive birds experience significant disease following infection with avian malaria, with reports as early as 1905 of die-offs from infection with *Plasmodium* in Gray Partridges (*Perdix perdix*) that were imported from Hungary and released in France (Garnham 1966). Despite this lengthy history, the number of reports of large-scale epizootics from avian malaria over the past 100 years are surprisingly limited, with most associated with wild Ciconiiformes in Venezuela (Gabaldon and Ulloa 1980), captive penguins (Fix et al. 1988), and native Hawaiian forest birds (Warner 1968).

There has been a recent renaissance in the use of prevalence data on hematozoan infections in birds to investigate ecological and evolutionary hypotheses about sexual selection and the physiological costs of parasitism in wild bird populations (Hamilton and Zuk 1982; Kilpatrick et al. 2006; Gilman et al. 2007). Some of this work is based on the use of molecular methods to diagnose very low intensity infections and track host specificity and geographic distribution of mitochondrial lineages of these parasites (Ricklefs et al. 2005). These new tools are leading to fundamental revisions in how we define species of *Plasmodium* and will play

an important role in assessing their impact on wildlife populations.

DISTRIBUTION

The species of *Plasmodium* that infect birds have a cosmopolitan distribution and are found in all major zoogeographic regions of the world with the exception of Antarctica, where mosquito vectors responsible for their transmission do not occur. Reports of *Plasmodium* from the Australian region are notably fewer than others, but it is not clear whether this is because this region has not been adequately sampled or whether it reflects a true distributional anomaly (Bennett et al. 1993; Valkiūnas 2005).

Seven species of *Plasmodium* have a cosmopolitan distribution and broad host range, with reports from as few as 67 species of avian hosts for *P. elongatum* to as many as 419 different species of birds for *P. relictum* (Bennett et al. 1993; Valkiūnas 2005). *Plasmodium relictum* and *P. circumflexum* have the broadest geographic distribution and are reported from the Nearctic, Palearctic, Oriental, Ethiopian, Neotropical, and Australian regions. *Plasmodium vaughani*, *P. cathemerium*, *P. nucleophilum*, *P. rouxi*, and *P. elongatum* have been reported from all regions with the exception of the Australian region (Bennett et al. 1993).

HOST RANGE

Infections with *Plasmodium* have been reported in birds from all avian orders with the exception of the Struthioniformes (ostriches), the Coliiformes (mousebirds), and the Trogoniformes (trogons and quetzals), but only about half of all avian species have been examined for these parasites. The greatest diversity of species of *Plasmodium* is recorded from the Galliformes, Columbiformes, and Passeriformes (Valkiūnas 2005). Important resources for locating host records and early literature on *Plasmodium* infections in wild birds have been prepared by the International Reference Centre for Avian Hematozoa (Herman et al. 1976; Bennett et al. 1981, 1982; Bishop and Bennett 1992).

Plasmodium relictum has one of the widest host ranges of the avian plasmodia, occurring naturally in 70 different avian families. The relatively broad host range of most species of *Plasmodium* from birds is considered to be characteristic of the avian species of this genus, but exceptions are common. Based on identifications made by traditional morphological methods, some species appear to have very restricted host distributions in wild populations. For example, *Plasmodium hermani* and *Plasmodium kempi* have been described from domestic and Wild Turkeys (*Meleagris gallopavo*) in North America, yet are different enough in morphological features to be described as separate species. *Plasmodium hermani* has also been found in Northern Bobwhite (*Colinus virginianus*) from the same habitats as Wild Turkeys in Florida, USA (Forrester et al. 1987), but prevalence in other species of wild birds from the same habitats is not known. While *P. kempi* is capable of infecting species of Galliformes and Anseriformes in the laboratory, Wild Turkeys are the only known natural host of this parasite (Christensen et al. 1983).

Recent application of molecular methods to screen avian hosts has revealed a far greater complexity of genetic lineages of *Plasmodium* and the closely related genus *Haemoproteus* that are currently difficult to relate to more traditional morphological species (Bensch et al. 2004). Multiple lineages can occur in the same host individual, and their occurrence in species from a wide range of avian orders, families, and species is much broader than previously recognized (Fallon et al. 2005; Ricklefs et al. 2005; Szymanski and Lovette 2005).

ETIOLOGY

Members of this genus are classified as members of the phylum Apicomplexa, class Aconoidasida, order Haemospororida, family Plasmodiidae and are defined primarily by their intraerthrocytic development and asexual reproduction (merogony, also called schizogony) in the circulating blood cells (Peirce 2000). All members of this genus produce prominent golden-brown or black pigment granules from digestion of host hemoglobin. The species of *Plasmodium* that infect birds are divided into five subgenera based on morphology of circulating gametocytes and meronts and on preference for mature or immature erythrocytes (Table 3.1; Figure 3.1; Valkiūnas 2005). Peirce and Bennett (1996) recognize a sixth subgenus among the avian parasites, *Plasmodioides*, that was erected for a single species, *Fallisia neotropicalis*, from pigeons and Ciconiiformes in Venezuela (Gabaldon et al. 1985). This unusual avian parasite lacks pigment granules in all stages of development and develops exclusively in circulating leukocytes and thrombocytes. Peirce and Bennett (1996) argue that similarities in life history characteristics justify including this parasite among the avian malarial parasites as a species of *Plasmodium*, but most workers now place this subgenus in the family Garniidae (genus *Fallisia*) with reptilian blood parasites that also undergo merogony in circulating leukocytes (Valkiūnas 2005).

Species of *Plasmodium* are further distinguished by host range, vectors, and developmental characteristics of exoerythrocytic tissue stages. More than 40

Table 3.1. Subgenera and species of avian *Plasmodium* and characteristics of erythrocytic stages of development.

Subgenus	Characteristics	Species
Haemamoeba	Gametocytes round and exceed size of host cell nucleus Mature parasites displace host cell nucleus Meronts present in mature erythrocytes	*Plasmodium relictum* *Plasmodium subpraecox* *Plasmodium cathemerium* *Plasmodium gallinaceum* *Plasmodium matutinum* *Plasmodium lutzi* *Plasmodium giovannolai* *Plasmodium griffithsi* *Plasmodium tejerai* *Plasmodium coturnixi* *Plasmodium parvulum*
Giovannolaia	Gametocytes elongate Mature parasites do not displace host cell nucleus Meronts present in mature erythrocytes Meronts larger than erythrocyte nucleus, with plentiful cytoplasm	*Plasmodium fallax* *Plasmodium circumflexum* *Plasmodium polare* *Plasmodium lophurae* *Plasmodium durae* *Plasmodium pedioecetae* *Plasmodium pinottii* *Plasmodium formosanum* *Plasmodium gundersi* *Plasmodium anasum* *Plasmodium garnhami* *Plasmodium hegneri* *Plasmodium octamerium* *Plasmodium gabaldoni* *Plasmodium leanucleus*
Novyella	Gametocytes elongate Mature parasites do not displace host cell nucleus Meronts present in mature erythrocytes Meronts smaller than erythrocyte nucleus, without noticeable cytoplasm	*Plasmodium vaughani* *Plasmodium columbae* *Plasmodium rouxi* *Plasmodium hexamerium* *Plasmodium nucleophilum* *Plasmodium dissanaikei* *Plasmodium paranucleophilum* *Plasmodium bertii* *Plasmodium kempi* *Plasmodium forresteri* *Plasmodium ashfordi*
Bennettinia	Gametocytes, round or oval, do not exceed size of host cell nucleus and stick to host nucleus Meronts present in mature erythrocytes Meronts round with scant cytoplasm and stick to host nucleus	*Plasmodium juxtanucleare*
Huffia	Gametocytes elongate Mature parasites do not displace host cell nucleus Meronts variable in form and size Meronts present in circulating erythrocyte precursors	*Plasmodium elongatum* *Plasmodium huffi* *Plasmodium hermani*

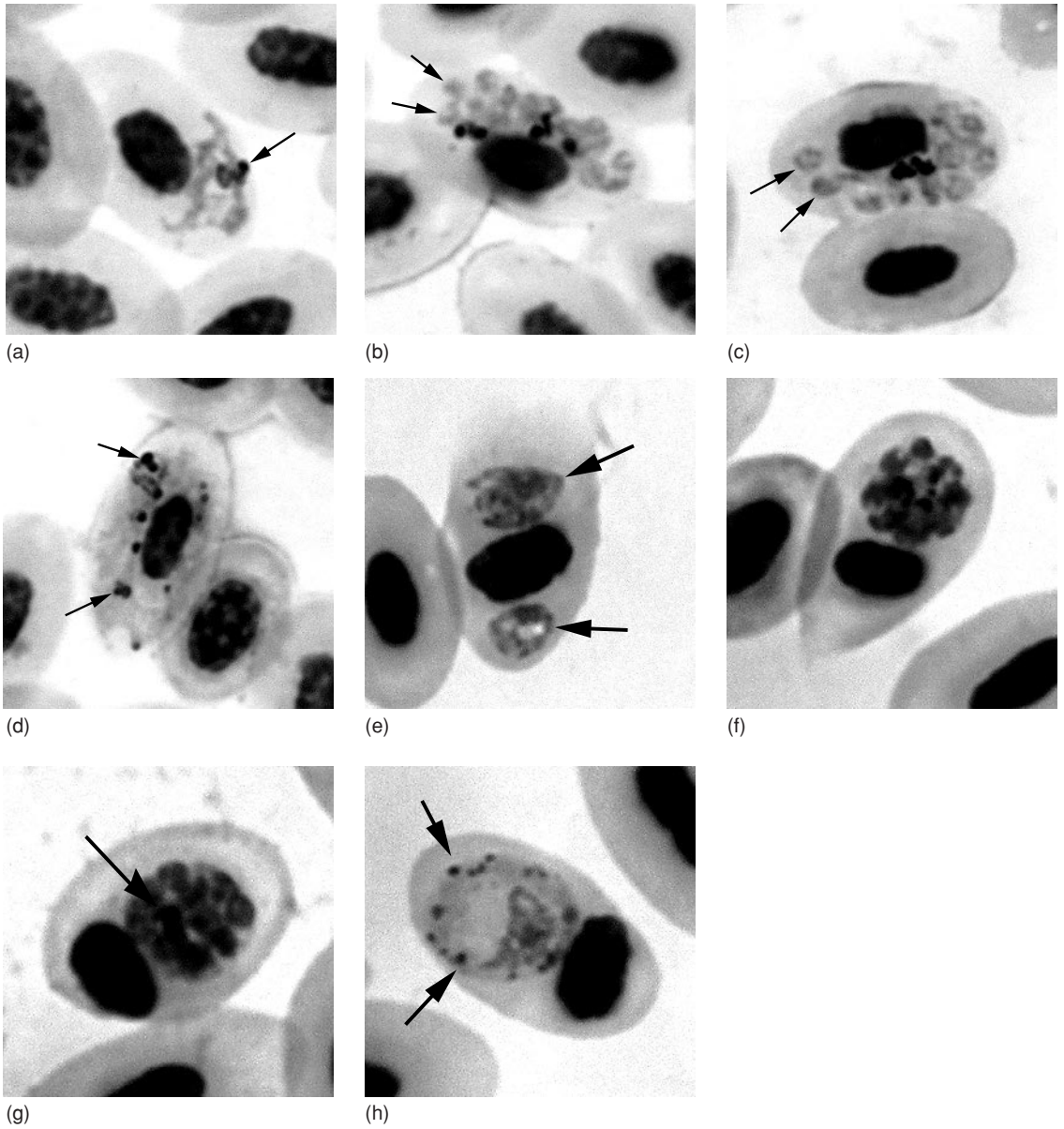

Figure 3.1. Erythrocytic stages of *Plasmodium circumflexum* (subgenus *Novyella*) (a–d) and *Plasmodium relictum* (subgenus *Haemamoeba*) (e–h). Note elongated shape of meronts (b, c) and gametocyte that encircles the host erythrocyte nucleus (d). The latter is characteristic of species of *Plasmodium* in the subgenus *Novyella*. Note shift in host erythrocyte nuclei (e–h) and round shape of gametocyte (h) that is characteristic of species of *Plasmodium* in the subgenus *Haemamoeba*. (a) Trophozoite. Note cluster of pigment granules (arrow). (b) Mature meront. Individual merozoites (arrows) are evident. (c) Mature meront. Note individual merozoites (arrows). (d) Gametocyte. Gametocyte surrounds the host erythrocyte nucleus, filling the erythrocyte cytoplasm. Pigment granules (arrows) are scattered through the parasite cytoplasm. (e) A pair of trophozoites (arrows). (f) Mature meront. Developing merozoites surround a central mass of pigment. (g) Mature meront. Developing merozoites surround a central mass of pigment (arrow). (h) Gametocyte. Note round shape, displaced host erythrocyte nucleus, and scattered pigment granules (arrows).

species are currently recognized, but this number is in a continual state of flux as existing species are synonomized as more information is learned about their biological characteristics and new species are described (Table 3.1).

The recent application of molecular methods to the taxonomy of this group has identified a bewildering array of lineages that are currently defined by sequence of mitochondrial genes (Bensch et al. 2004). In most cases, we know nothing about their erythrocytic morphology, natural vectors, or other life history characteristics and are only just beginning to link this information to the more traditional morphological and biological definition of individual species (Valkiūnas et al. 2007). Recent efforts to combine molecular data with life history information from members of five subgenera (*Haemamaoba*, *Huffia*, *Bennettinia*, *Novyella*, and *Giovannolaia*) indicate that the very distinctive characteristics of the subgenera *Haemamoeba* (large, round gametocytes and prominent host nucleus displacement), *Huffia* (predilection for immature erythrocytes), and *Bennettinia* (unusual morphology of the erythrocytic and sporogonic stages) are consistent with monophyletic origins for each of these subgenera. By contrast, *Novyella* and *Giovannolaia* form a clade composed of representatives from both subgenera, indicating that the less distinctive morphology of these parasites appears to be more plastic over evolutionary time (Martinsen et al. 2008). These findings suggest that some of the key morphological features used by parasitologists to distinguish these subgenera may not reflect true phylogenetic relationships (Martinsen et al. 2008). It is clear that our understanding of the taxonomy and phylogenetics of these parasites is rapidly evolving, and their classification will likely undergo further revision in future years.

EPIZOOTIOLOGY

Much of what we know about the detailed life cycle of species of *Plasmodium* from birds is based on a series of classic experiments by Clay Huff and coworkers with *Plasmodium gallinaceum*. In these studies, chickens and other birds were exposed to infective mosquito bites and examined at sequential time intervals to determine the location and morphology of the parasites (Huff and Coulston 1944; Huff 1951). These studies have provided us with specific details about how some of these parasites develop, but variations in the life cycle have been documented among other species of *Plasmodium* and further studies are needed.

The life cycle of *P. gallinaceum* begins when infective sporozoites are inoculated by a mosquito vector into a susceptible host (Huff and Coulston 1944). Sporozoites invade macrophages and fibroblasts near the site of the mosquito bite and undergo an initial generation of asexual reproduction (merogony) as cryptozoites. These are relatively small in diameter and mature in approximately 36–48 h to release ovoid merozoites that invade cells of the lymphoid–macrophage system in brain, spleen, kidney, lung, and liver tissue to begin a second generation of merogony as metacryptozoites. Metacryptozoites mature and release merozoites that are capable of invading circulating erythrocytes and capillary endothelial cells of the major organs. The first two generations of merogony are referred to as the preerythrocytic stages of infection. Merozoites that continue with a third generation of merogony in stationary tissues of the host are called phanerozoites. Once they invade capillary endothelial cells and begin to reproduce by asexual merogony, they are referred to as exoerythrocytic meronts. Merozoites released from exoerythrocytic meronts can either invade circulating erythrocytes or reinvade endothelial cells to continue additional generations of merogony in stationary tissues. The exoerythrocytic meronts that occur in capillary endothelial cells are oval, elongate, or branching and similar in morphology to thin-walled meronts of *Haemoproteus* (Chapter 2). They are significantly larger than the preerythrocytic meronts and may contain hundreds of nuclei (Garnham 1966).

Merozoites that invade the circulating erythrocytes undergo merogony and develop within 24–48 h into either mature meronts containing 8–32 ovoid merozoites or gametocytes that are infective to mosquito vectors (Table 3.1). Depending on the species of *Plasmodium*, meronts may be either round or elongate and produce numbers of merozoites that may be characteristic for particular species. Merozoites typically bud from a central residual mass and destroy their host erythrocyte when they are released. By contrast, gametocytes are elongate or round and have a single nucleus. The male gametocytes (microgametocytes) typically stain pink with Giemsa stain, while female gametocytes (macrogametocytes) stain pale blue.

During growth in the erythrocyte, the parasites ingest host erythrocyte cytoplasm through a specialized structure known as a cytostome and digest host hemoglobin within one or more food vacuoles scattered throughout the cytoplasm of the parasite. Malarial pigment or hematozoin is produced as a by-product of the digestion of hemoglobin and may appear as golden-brown or black granules in the parasite cytoplasm. Clear food vacuoles with one or more pigment granules may be visible by light microscopy, depending on size of the vacuoles. Merogony may continue indefinitely in the circulating erythrocytes, and evidence suggests that merozoites from some erythrocytic meronts can reinvade stationary tissues and continue development as phanerozoites (Garnham 1966).

Unlike *P. gallinaceum* (subgenus *Haemamoeba*), *P. elongatum* and other species in the subgenus *Huffia* do not develop in capillary endothelial cells of the major organs, but instead undergo exoerythrocytic merogony in hematopoietic tissues of the host (Garnham 1966). Specific details about preerythrocytic stages of development are not known.

Gametocytes of all species of avian *Plasmodium* remain in the circulation and do not continue development until they are ingested by an arthropod vector. Once in the midgut of a suitable mosquito vector, they leave their host cells and undergo gametogenesis to form gametes. Male gametocytes undergo a process call exflagellation to produce up to eight, flagellated microgametes. One microgamete will fertilize a macrogamete and within 24 h a motile zygote develops, which is capable of penetrating the midgut wall and beginning development as an oocyst under the basal membrane of the mosquito midgut. These initial stages of gametogenesis and fertilization exhibit little or no host specificity for mosquito vectors and can be completed in vitro. It is only during invasion of the peritrophic membrane that surrounds the blood meal and subsequent penetration of the midgut epithelium that blocks in development of particular species of malaria, in particular mosquito hosts, can occur (Michel and Kafatos 2005).

Oocysts undergo a type of asexual reproduction called sporogony and eventually produce thousands of sporozoites through a process of budding from multiple residual masses or sporoblasts. Oocysts mature within approximately 7 days after reaching a diameter of approximately 40 μm, depending on ambient temperature, and rupture to release sporozoites into the hemoceol of the mosquito. Sporozoites move via the hemocoel to the salivary glands, penetrate the glandular cells, and eventually gain access to the salivary ducts. When a mosquito takes a blood meal, these pass with the saliva into a new avian host to initiate a new infection.

Birds typically undergo an acute phase of infection where parasitemia increases steadily to reach a peak in numbers, called the crisis, approximately 6–12 days after parasites first appear in the blood. This is followed by a rapid decline in intensity of infection to chronic levels as the host immune system begins to bring the infection under control. Chronic infections most likely persist for the lifetime of infected birds, and both circulating parasites and persistent exoerythrocytic meronts can serve as a source for recrudescing infections (Manwell 1934; Bishop et al. 1938; Garnham 1966).

More than 60 different species of culicine and anopheline mosquitoes are capable of supporting experimental development of a variety of species of *Plasmodium* from avian hosts (Huff 1965), but surprisingly, few natural mosquito vectors are known (Table 3.2). For example, more than 20 species of anopheline and culicine mosquitoes in four different genera (*Culex*, *Aedes*, *Culiseta*, and *Anopheles*) are capable of transmitting *P. relictum* in the laboratory, but only three—*Culex quinquefasciatus*, *Culex tarsalis*, and *Culex stigmatasoma*—are proven natural vectors of *P. relictum* in California and Hawaii (Reeves et al. 1954; LaPointe et al. 2005).

After the initial acute phase of infection, intensity appears to be influenced by the complex interplay of host immunity, seasonal changes in photoperiod, and hormonal changes associated with reproduction. As has been described for other hematozoan parasites (Chapters 2 and 4), an increase in intensity of infection coincides with the breeding season when populations of blood-sucking insects typically increase, and recently fledged susceptible birds are increasing in the population (Atkinson and van Riper 1991; Valkiūnas et al. 2004). Termed the "spring relapse," the increase in numbers of parasites in the peripheral circulation can be triggered by corticosterone (Applegate and Beaudoin 1970), increases in photoperiod, and subsequent physiological changes in levels of hormones such as melatonin that regulate circadian rhythms (Valkiūnas et al. 2004). Many of the same factors that affect intensity of infection with *Haemoproteus* (Chapter 2) probably affect intensity of infection with *Plasmodium*. These include stress-mediated changes in the immune system that are associated with reproductive effort (Siikamäki et al. 1997), food availability (Appleby et al. 1999), concomitant infection with other parasites (Wright et al. 2005), and exposure to predators (Navarro et al. 2004).

While it is clear that most transmission of avian *Plasmodium* takes place during the spring and summer months in temperate climates, relatively little is known about dynamics of infection in tropical parts of the world. In Hawaii, transmission of *P. relictum* at lower elevations can take place throughout the year (Woodworth et al. 2005), but is more seasonal at higher elevations where both temperature and rainfall have significant effects on vector populations (Ahumada et al. 2004). By contrast, transmission of *P. hermani* in Wild Turkeys in subtropical Florida is limited primarily to late summer and early fall when populations of the primary vector, *Culex nigripalpus*, reach a peak. As is the case with *Haemoproteus* (Chapter 2), both the spatial and seasonal patterns of transmission depend on availability of suitable mosquito vectors and susceptible avian hosts. Among migratory species, recent evidence indicates that transmission of some species of *Plasmodium* and other haemosporidian parasites can occur on both the breeding and the wintering grounds,

Table 3.2. Proven and suspected natural vectors of species of *Plasmodium* from birds, based on demonstration of oocysts or sporozoites from wild mosquitoes or transmission by wild-captured mosquitoes.

Parasite species	Locality	Mosquito vector	Reference
Plasmodium relictum	California, USA	*Culex stimatosoma*	Reeves et al. (1954)
	California, USA	*Culex tarsalis*	Reeves et al. (1954)
	Hawaii, USA	*Culex quinquefasciatus*	LaPointe et al. (2005) and Woodworth et al. (2005)
Plasmodium gallinaceum	Sri Lanka	*Mansonia crassipes*	Niles et al. (1965) and Garnham (1966)
Plasmodium circumflexum	Sri Lanka	*Mansonia crassipes*	Niles et al. (1965) and Garnham (1966)
	New Brunswick, Canada	*Culiseta morsitans**	Meyer et al. (1974)
Plasmodium rouxi	Algeria	*Culex pipiens*	Sergent et al. (1928) and Garnham (1966)
Plasmodium juxtanucleare	Malaysia	*Culex sitiens*	Bennett et al. (1966)
		Culex annulus	Bennett and Warren (1966)
	Brazil	*Culex saltanensis*	Lourenco-de-Oliveira and de Castro (1991)
Plasmodium hermani	Florida, USA	*Culex nigripalpus*	Forrester et al. (1980)
Plasmodium elongatum	Maryland, USA	*Culex pipiens**	Beier and Trpis (1981)
		*Culex restuans**	Beier and Trpis (1981)
Plasmodium (*Novyella*) sp.	Venezuela	*Aedeomyia squamipennis*	Gabaldon et al. (1977) and Gabaldon and Ulloa (1980)
Plasmodium (*Giovannolaia*) sp.	Venezuela	*Aedeomyia squamipennis*	Gabaldon et al. (1977) and Gabaldon and Ulloa (1980)

Note: Numerous other species of mosquitoes are capable of supporting development of avian species of *Plasmodium* under laboratory conditions (Huff 1965), but few studies have isolated *Plasmodium* from naturally infected vectors or linked these with demonstrated transmission in the wild.

*Sporozoites or oocysts of undetermined species were demonstrated in wild mosquitoes; laboratory susceptibility was confirmed.

leading to increases in parasite dispersal (Pérez-Tris and Bensch 2005; Hellgren et al. 2007).

Both intrinsic and extrinsic factors affect the distribution and prevalence of the closely related genus *Haemoproteus* (Chapter 2). Many of these same factors also determine the prevalence of *Plasmodium*, but this has not been examined in as much detail. Based on surveys by microscopy, prevalence of *Plasmodium* is four to five times lower than either *Haemoproteus* or *Leucocytozoon*, with an overall prevalence of less than 4% in a sample of over 2,000 birds from North America (Greiner et al. 1975). Prevalence of *Plasmodium* differed in specific physiographic regions of the continent, ranging as high as almost 10% in the southeastern US to less than 1% in the arctic barrens (Greiner et al. 1975). Very low prevalences of *Plasmodium* relative to *Haemoproteus* and *Leucocytozoon* may largely be a sampling artifact because very low intensity chronic infections are extremely difficult to detect by microscopy. Prevalence of *Plasmodium* is much higher when more sensitive diagnostic methods are used, such as those based on the polymerase chain reaction (PCR). For example, prevalence of *Plasmodium* in forest birds from American Samoa is 1% by microscopy, but approximately 60% by PCR amplification of parasite ribosomal genes (Jarvi et al. 2003; Atkinson et al. 2006).

Given their higher sensitivity, molecular methods may be valuable for investigating the effects of host behavior and ecology on prevalence of infection. In a large study of host and parasite community relationships in southern Missouri, USA, prevalence was weakly correlated with host body mass, but not with foraging stratum, nest height, nest type, plumage brightness, sexual dichromatism, age, or sex (Ricklefs et al. 2005). Significant relationships may have been obscured, however, by analysis of multiple parasite lineages that may differ in specific life history

characteristics. In more intensive studies of individual species of *Plasmodium* in defined host populations, prevalence may differ by both age and sex. For example, prevalence of infection with *P. circumflexum* and *P. cathemerium* is significantly higher in adults rather than juvenile Red-winged Blackbirds (*Agelaius phoeniceus*; Herman 1938), and differences in prevalence of *Plasmodium* by sex have been reported in other studies of this host species (Weatherhead and Bennett 1991).

The potential confounding effects of simultaneous infection with other haemosporidian parasites may also influence prevalence of *Plasmodium* by maintaining infections at higher frequencies than might be expected. For example, specific *Mhc* alleles that seem to be associated with susceptibility to *Plasmodium* may be maintained in a population of House Sparrows (*Passer domesticus*) because they confer resistance to a coinfecting strain of *Haemoproteus* (Loiseau et al. 2008).

CLINICAL SIGNS

Infections with *P. relictum* (canaries, Hawaiian honeycreepers, penguins), *P. gallinaceum* (domestic chickens), *P. juxtanucleare* (domestic chickens), *P. elongatum* (penguins), and *P. durae* (domestic turkeys) can be extremely pathogenic during acute phases of infection in their respective hosts (Garnham 1966; Stoskopf and Beier 1979; Huchzermeyer 1993a; Yorinks and Atkinson 2000; Williams 2005). Infected birds are typically anemic, lethargic, anorexic, and have ruffled feathers. Hematocrits may fall by more than 50% (Figure 3.2). Domestic chickens infected with *P. gallinaceum* and *P. juxtanucleare* have been described as lethargic, having pale combs, green droppings, diarrhea, and partial or total paralysis (Garnham 1966). Young turkeys with infections of *P. durae* exhibit few clinical signs until immediately before death, when severe convulsions may occur (Garnham 1966). Adult turkeys typically become lethargic, anorexic, and often develop right pulmonary hypertension as a consequence of hypoxic pulmonary arterial hypertension (Huchzermeyer 1988). Adult birds may also develop edematous legs and gangrene of the wattles. Cerebral capillaries may be blocked by developing exoerythrocytic meronts, and infected birds may exhibit neurological signs and paralysis before death (Garnham 1966).

During the crisis, when peripheral parasitemias reach their peak, chickens infected with *P. gallinaceum* have reduced plasma albumin and α_2-globulin as well as significant increases in γ_1- and γ_2-globulin (Williams 2005). These changes coincide with significant increases in plasma total protein and aspartate aminotransferase, glutamate dehydrogenase, and γ-glutamyltransferase and a decrease in creatinine that likely reflect tissue damage caused by developing both erythrocytic and exoerythrocytic parasites (Williams 2005). Increases in white blood cell counts, relative and absolute lymphocytosis, and total plasma solids have been documented in Hawaiian Crows (*Corvus*

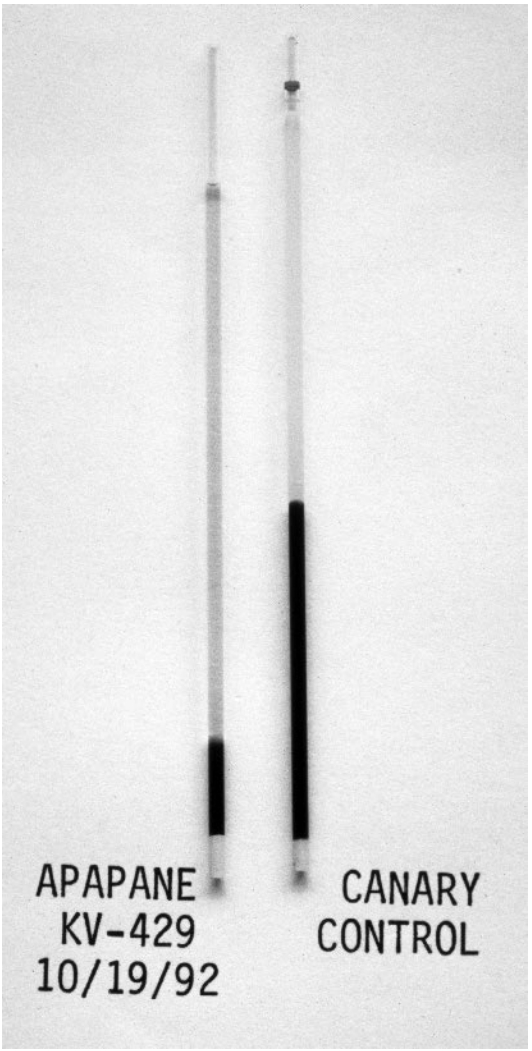

Figure 3.2. Hematocrit for a Wild Apapane (*Himatione sanguinea*) (left) with an acute natural infection with *Plasmodium relictum*. The hematocrit from an uninfected control canary (right) illustrates the severity of the anemia. Birds with acute infections of this intensity are rarely captured with mist nets in the wild.

hawaiiensis) and penguins with acute infections with *P. relictum* (Graczyk et al. 1994c; Massey et al. 1996). Hematological changes are much less evident in birds with chronic infections (Ricklefs and Sheldon 2007).

PATHOLOGY AND PATHOGENESIS

Avian malaria is primarily a disease of the blood and reticuloendothelial system, and the progress of the disease and clinical signs closely parallel increases in the number of parasites in the peripheral circulation (van Riper et al. 1994). In detailed studies of *P. gallinaceum* in experimentally infected chickens, clinical signs first become evident from 5 to 7 days after inoculation of infected blood (Williams 2005). These correspond to rapid increases in peripheral parasitemia and declines in hematocrit (Figure 3.3). Hemolysis of both infected and uninfected erythrocytes and catabolism of hemoglobin leads to production of excess biliverdin, which is excreted in the feces (Williams 1985). Infected birds begin to excrete green feces approximately 4 days after infection. During phase I, lasting only a few hours, feces are normal in form with green pigment confined to the fecal portion of the dropping. Thin, mucoid, brilliant green diarrhea develops by day 5 (phase II), which persists about 2 days among birds that survive infection. During phase III, birds are recovering from infection, and green coloration of the droppings is intermediate in intensity between that observed during phase I and phase II. Droppings lose all green color by the time that parasitemia becomes undetectable (Williams 2005).

It is not clear whether acute infections with *Plasmodium* cause the febrile paroxysms in birds that are so characteristic of human malarial infections. Increases in cloacal temperature have been measured during acute phases of infection with *P. gallinaceum* in chickens (Williams 2005). As is the case with human infections, the febrile period was relatively short-lived and closely paralleled increases in peripheral parasitemia. Following the crisis, cloacal temperatures fell and then remained below normal for several days (Figure 3.3).

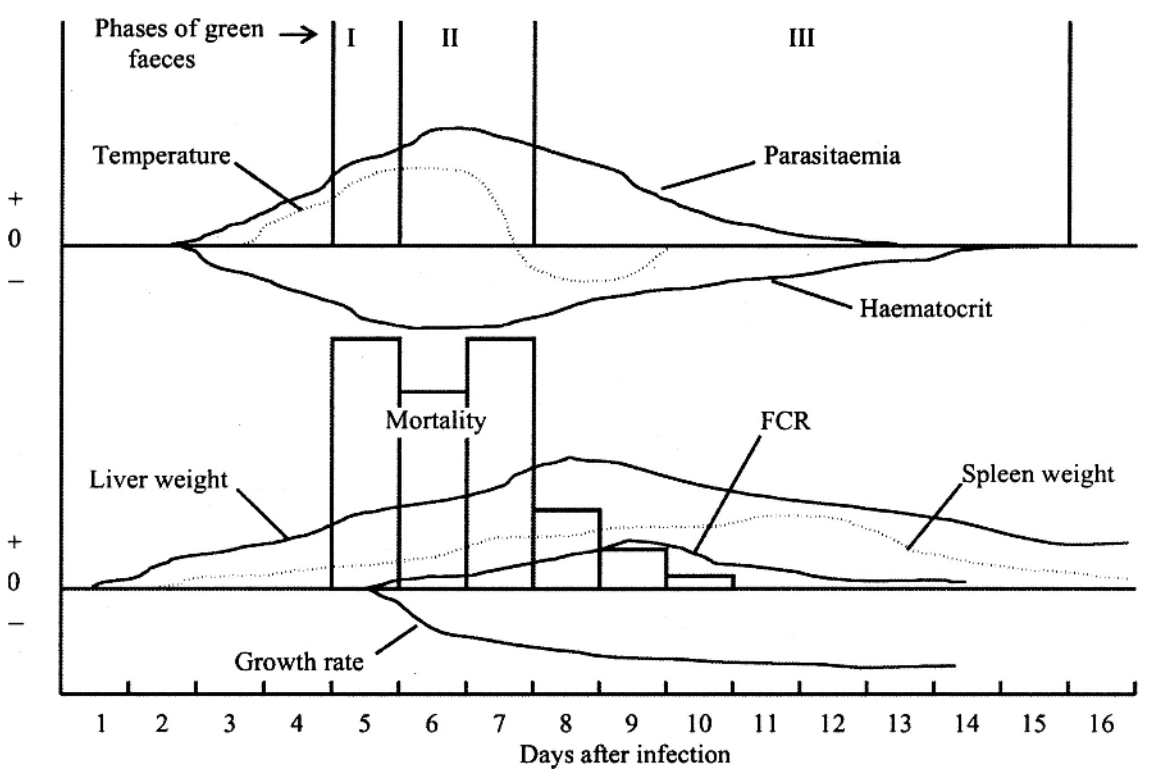

Figure 3.3. Relative timing of clinical signs of *Plasmodium gallinaceum* in domestic chickens following a blood-induced infection. Lines represent deviations from baseline conditions in healthy birds. FCR, food conversion ratio. Reproduced from Williams (2005), with permission of Taylor & Francis Ltd. (http://www.informaworld.com).

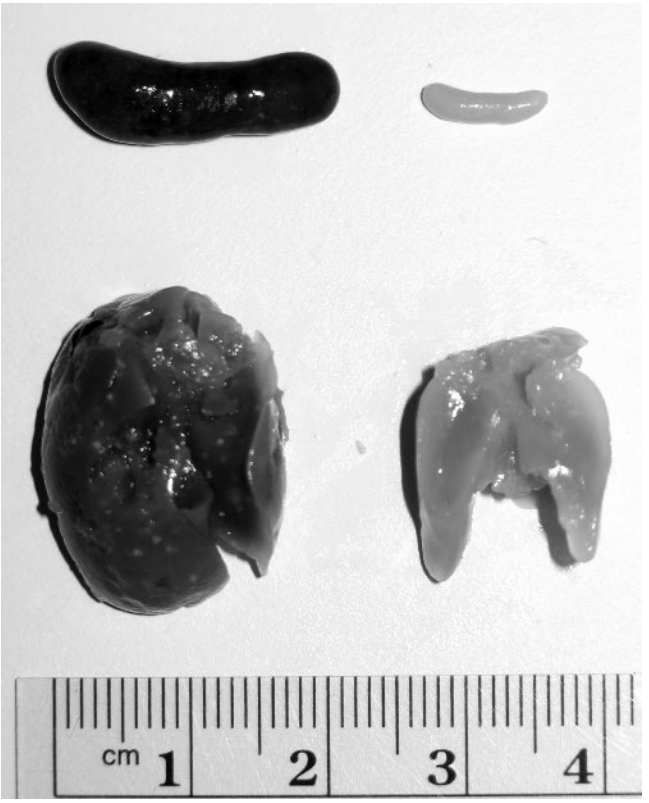

Figure 3.4. Livers and spleens from an uninfected control canary (right) and a canary with an experimental acute infection with *Plasmodium relictum* (left). Infected liver (bottom left) is enlarged, has rounded borders, is discolored from deposition of malarial pigment in tissue macrophages, and has multifocal areas of necrosis. Infected spleen (top left) is similarly enlarged and discolored from deposition of malarial pigment in tissue macrophages. Tissue has been fixed in 10% buffered formalin.

By contrast, canaries infected with *P. relictum* have significant declines in core body temperature during acute phases of infection and appear to lose the ability to thermoregulate (Hayworth et al. 1987).

The hallmark gross lesions produced by acute infections with *Plasmodium* include thin, watery blood, and enlargement and discoloration of the liver and spleen by deposition of malarial pigment in tissue macrophages (Figure 3.4). Enlargement of these organs is due to hypercellularity and increased phagocytic activity of macrophages rather than edema (Al-Dabagh 1966). Development of gross lesions closely corresponds to a steady increase in peripheral parasitemia, intravascular hemolysis of infected erythrocytes as meronts mature, phagocytosis of parasitized erythrocytes, and increased fragility of unparasitized erythrocytes (Al-Dabagh 1966; Seed and Kreier 1972; van Riper et al. 1994; Williams 2005). Regenerative, hemolytic anemia is associated with a drop in erythrocyte counts, replacement with immature erythrocytes, and drops in hemoglobin concentration that peak during the crisis (Figure 3.5). Anoxia and intravascular agglutinations of erythrocytes ("sludging" of blood) may lead to damage of endothelial cells lining the capillaries (Al-Dabagh 1966). Deposition of malarial pigment in macrophages of various organs, particularly liver and spleen, as infected cells are removed from the circulation can be extensive. In intense fatal infections, thrombi or emboli can form in some organs, particularly the spleen. Secondary shock may also occur during the terminal stages of some acute infections, resulting from destruction of large numbers of infected and uninfected erythrocytes. Capillaries and venules may be dilated and exhibit increased permeability, edema, and stasis of blood flow. Hemorrhage may be evident within the capillaries. Lowered blood pressure, lowered blood volume, disturbed fluid balance, increased coagulation times, and increased levels of potassium may also be evident in severe infections (Al-Dabagh 1966).

Infections with *P. cathemerium* produce inflammatory myopathy in skeletal muscle of experimentally infected canaries. This is characterized by degeneration of capillaries and muscle fibers and presence of mononuclear cell infiltrates. Carmona et al. (1996) suggest that this may be related to obstruction of capillaries

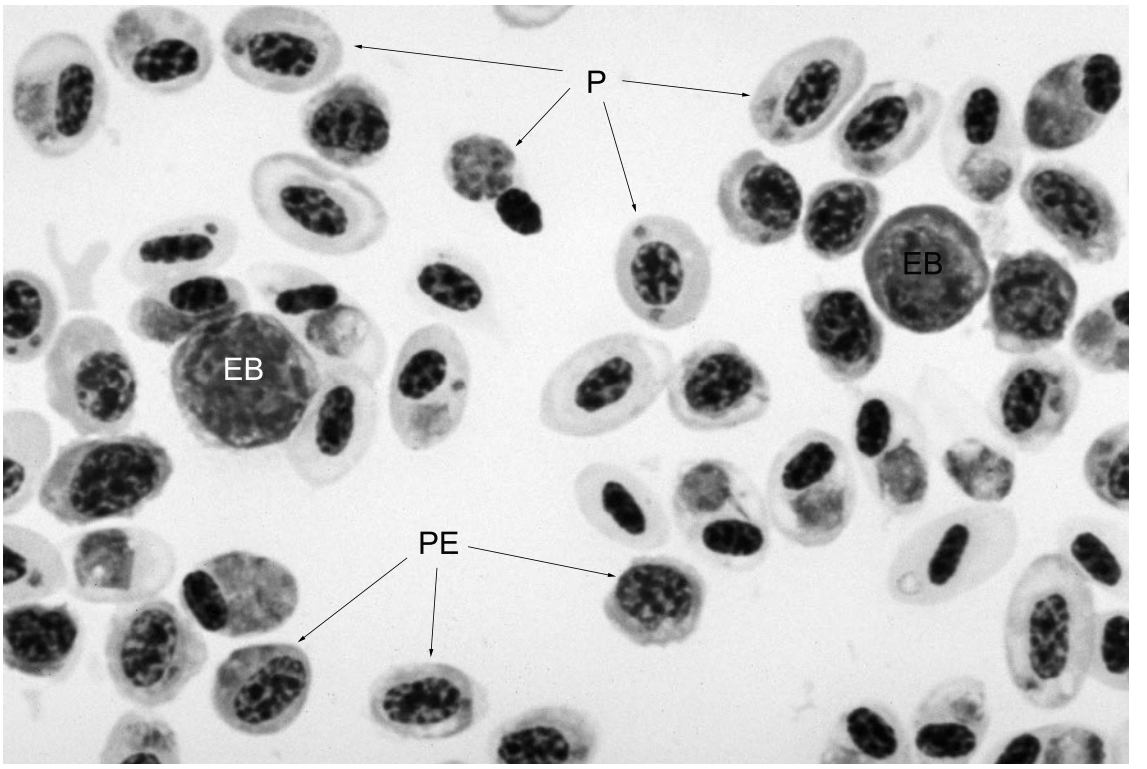

Figure 3.5. Blood smear from an liwi (*Vestiaria coccinea*) with an experimental infection with *Plasmodium relictum*. The normal cellular makeup of the blood is profoundly altered, with mature erythrocytes being replaced by erythroblasts (EB) and early polychromatic erythrocytes (PE). P, parasitized erythrocytes (Atkinson et al. 1995).

by infected erythrocytes. Anemia may also lead to circulatory deficiency that is compensated in part by increased cardiac output and dilation and hypertrophy of heart muscle (Al-Dabagh 1966).

While little or no host response is evident around preerythrocytic meronts of *Plasmodium*, the exoerythrocytic meronts of some species, for example, *P. gallinaceum* and *P. durae*, may partially or completely block capillaries, leading to leakage of plasma proteins, edema, and hemorrhage. These lesions may occur in the heart, lungs, renal glomeruli, and brain. When they occur in the brain, neurologic symptoms may appear and death can be sudden.

There is a clear association between the severity of disease and dose. This has been demonstrated experimentally with both blood-induced infections (Permin and Juhl 2002) and sporozoite-induced infections (Atkinson et al. 1995). Birds exposed to higher numbers of infective sporozoites have higher parasitemias, more severe gross and microscopic lesions, and higher mortality (Atkinson et al. 1995, 2000).

DIAGNOSIS

The gold standard for diagnosis of *Plasmodium* is a Giemsa-stained thin blood smear where it is possible to demonstrate the presence of erythrocytic meronts and gametocytes with prominent golden-brown or black pigment granules. Individual species are traditionally defined by size and shape of intraerythrocytic gametocytes and meronts (Table 3.1; Figure 3.1), number of merozoites produced by mature meronts, changes in morphology of the host erythrocyte, and other biological characteristics such as host range, susceptibility to species of mosquitoes, morphology, and location of exoerythrocytic meronts (Garnham 1966; Valkiūnas 2005). Since most identifications are made from blood smears, life history characteristics may be unknown, and it becomes essential to be able to find enough mature meronts and gametocytes on a smear to be able to make an accurate assessment of parasite morphology. Detailed keys and species descriptions have been recently revised by Valkiūnas (2005), and his monograph is currently the most

up-to-date resource for identifying species of avian *Plasmodium*.

Most infections of *Plasmodium* in wild birds are chronic, however, and intensity may be extremely low. In these cases, it may be impossible to identify parasites below level of subgenus. When erythrocytic meronts are not present, it may become difficult to distinguish gametocytes of *Plasmodium* from those of *Haemoproteus*, although gametocytes of *Haemoproteus* are often thicker and more robust than those of *Plasmodium*. The fact that species of *Plasmodium* have circulating meronts while species of *Haemoproteus* do not can be used to both isolate and identify an unknown species of *Plasmodium* if susceptible domestic or captive wild birds are available for experimental subinoculation of blood from the suspect bird. While it was common knowledge among early malariologists that *Plasmodium* can be passed to a new host by blood inoculation, Manwell and Herman (1935) and later Herman (1938) were the first to apply this method to diagnose infections with *Plasmodium* in wild birds. Blood from an infected host is passed by intravenous, intraperitoneal, or intramuscular inoculation into an uninfected host of the same species, and blood smears are prepared from the inoculated host for several weeks after injection. If the host is susceptible to the parasites, an acute phase infection will often result and meronts and gametocytes can be readily found for morphological analysis. When parasitemia is high, blood can be collected, treated with glycerin or dimethyl sulfoxide, aliquoted, and frozen in liquid nitrogen to create a frozen stabilite for further experimental studies (Garnham 1966).

Given the importance of morphological characters to identify species of *Plasmodium* from birds, their consistency and stability between hosts of different species is critical for making accurate identifications. Surprisingly, few studies have looked at this issue in detail. In one of the most widely cited examples, when *P. relictum* from Silver Gulls (*Larus novaehollandiae*) was passed by sporozoites to sparrows and canaries, merozoite, and gametocyte morphology changed significantly (Lawrence and Bearup 1961). In gulls, gametocytes were elongate and mature meronts had 10 merozoites. In sparrows, morphology was more typical of *P. relictum* and gametocytes were round or oval, and mature meronts had on average 14 merozoites. Other reports have documented changes in morphology when parasites are inoculated into atypical hosts (Garnham 1966) or when parasitemias are extremely high in immature erythrocytes (Laird and van Riper 1981). By contrast, other reports have documented relatively constant morphology in hosts from multiple avian species and orders (van Riper et al. 1986; Iezhova et al. 2005; Valkiūnas et al. 2007; C. T. Atkinson, unpublished observations). This issue clearly needs further study, and the relatively recent development of molecular methods to diagnose avian malaria with PCR primers to ribosomal and mitochondrial genes may help to resolve this problem. Despite their higher sensitivity, PCR methods may still miss infections that have extremely low parasitemias (Jarvi et al. 2002), although the recent application of real-time methods to malarial diagnostics may eventually solve these problems (Boonma et al. 2007).

Several recent sets of primers designed to amplify portions of the parasite mitochondrial genome can distinguish *Haemoproteus* and *Plasmodium* from *Leucocytozoon* (Hellgren et al. 2004) or all three genera from each other following restriction digests of PCR products (Beadell and Fleischer 2005). However, sequencing of PCR products is necessary for identifying individual parasite lineages and determining phylogenetic relationships. Since so few isolates of avian *Plasmodium* of known identity have been sequenced and typed, it is often not known how to relate unknown mitochondrial lineages to traditional morphological species. Recent rapid progress in the molecular diagnosis of avian species of *Plasmodium* may eventually make it possible to identify species based on mitochondrial lineage (Valkiūnas et al. 2007).

Plasmodium appears to be antigenically distinct from *Haemoproteus*, and crude antigen extracts have been used to develop an ELISA test for *P. relictum* in captive and wild penguins (Graczyk et al. 1994a, b). Standard immunoblotting techniques can also be used to identify antibodies to *Plasmodium* in wild and experimentally infected passerines (Atkinson et al. 2001). Although neither ELISA nor immunoblotting can distinguish species of *Plasmodium*, the techniques are useful for making diagnoses to level of genus in birds with low-intensity infections that may be missed by microscopy or PCR.

IMMUNITY

Birds infected with avian species of *Plasmodium* develop strong antibody and cell-mediated responses to erythrocytic parasites (van Riper et al. 1994), but appear to be unable to completely clear their infections. Limited evidence based on experimental studies in canaries (*P. relictum*), Hawaii Amakihi (*Hemignathus virens*) (*P. relictum*), and domestic turkeys (*P. hermani*) indicates that birds likely remain infected for life, but at chronic levels that stimulate immunity to reinfection with homologous strains of the parasite (Bishop et al. 1938; Jarvi et al. 2002; Young et al. 2004). This phenomenon, termed premunition, was recognized in the early part of the twentieth century (Hewitt 1940; Sergent and Sergent 1956). When birds with blood or sporozoite-induced infections are rechallenged, they

may have only brief, low-intensity increases in peripheral parasitemia (Hewitt 1940; Atkinson et al. 2001; Paulman and Mcallister 2005).

The persistence of subclinical infections may make birds vulnerable to the recrudescence of erythrocytic parasites if host immunity is compromised by stress or infection with other pathogens and provides an indirect measure of the cost of mounting an immune response. Experimental manipulation of clutch size led to increases in prevalence of *Plasmodium* in female Great Tits (*Parus major*) that laid more eggs, supporting the idea that there is a trade-off between the energetic costs of egg production and defense against parasites (Oppliger et al. 1996). Similarly, male Great Tits that expended extra energy to provision larger broods had a higher prevalence of malarial infection (Richner et al. 1995).

Exposure to other infectious diseases that compromise the immune system may also lead to recrudescing infections. When Wild Turkeys are exposed simultaneously or sequentially to turkeypox virus and *P. hermani*, both parasitemia and mortality are higher in 1-week-old poults infected with both agents than those exposed to either malaria or pox alone (Wright et al. 2005). These effects are less evident in older poults, suggesting that the host age may also play a role in pathogenesis of concomitant infections.

PUBLIC HEALTH CONCERNS

Avian species of *Plasmodium* do not infect humans, and infected birds pose no health risks to humans.

DOMESTICATED ANIMAL HEALTH CONCERNS

Domestic poultry are susceptible to several species of avian malaria, but their most significant effects occur outside of North America and Europe and specifically where wild reservoir hosts serve as sources of infection for domestic birds. *Plasmodium gallinaceum* is highly pathogenic in domestic chickens, particularly when European breeds are introduced to endemic areas in southeastern Asia, Malaysia, India, and Sri Lanka where the natural host is the Red Junglefowl (*Gallus gallus*; Garnham 1966). The distribution of the parasite in domestic chickens coincides with the geographic range of the natural host and has not expanded with the movement of domestic poultry to other parts of the world. *Plasmodium juxtanucleare* is also a significant pathogen in domestic chickens in South America, southern Africa, and southeastern Asia. Proven wild reservoirs of this species are found in India, Malaysia, South Africa, and Taiwan and include Red Junglefowl, Gray-winged Francolins (*Francolinus africanus*), and Chinese Bamboo-Partridges (*Bambusicola thoracicus*), but natural hosts are not known for other parts of its range (Garnham 1966; Fernando and Dissanaike 1975; Manwell et al. 1976; Earle et al. 1991).

Domestic turkeys are highly susceptible to *P. durae* in sub-Saharan Africa. This species is a parasite of wild francolins that infects domestic turkeys when wild reservoir hosts and vectors are present (Huchzermeyer 1993b). *P. durae* is highly pathogenic in domestic turkeys, and mortalities can be as high as 90% in young poults. Both *P. kempi* and *P. hermani* infect Wild Turkeys in North America, but have not reported to be a problem in domestic birds.

WILDLIFE POPULATION IMPACTS

There is relatively little evidence that species of avian *Plasmodium* are causes of major epizootic die-offs in their natural hosts. In a frequently cited example, high rates of transmission of species of *Plasmodium* from several subgenera have been documented in Venezuela among nesting Ciconiiformes, but clear evidence of malarial mortality in dead nestlings is not provided (Gabaldon and Ulloa 1980). In a thorough review of over 5,000 papers on avian blood parasites, Bennett et al. (1993) found that only about 4% reported mortality or pathogenicity in birds, with most dealing with domestic birds or birds in zoological collections.

Evidence is beginning to accumulate, however, that both direct and indirect effects of acute and chronic infections can have measurable impacts on the lifetime reproductive success of their avian hosts. In a study of singing behavior in White-crowned Sparrows (*Zonotrichia leucophrys oriantha*), song consistency was influenced by infection with *Plasmodium* and *Leucocytozoon*. Birds infected with *Plasmodium* also sang fewer songs following experimental playback of recorded songs (Gilman et al. 2007). This could have a significant impact on mate choice and reproductive success of infected males. Similarly, the behavioral effects of acute infections may lead to increased predation of infected hosts (Yorinks and Atkinson 2000; Møller and Nielsen 2007). These questions are just beginning to be explored in detail in ecological studies of wild birds, and the careful integration of both field and laboratory studies may lead to significant progress in our understanding of the more subtle costs of infection with these parasites.

The most significant reports of pathogenicity among species of *Plasmodium* that infect birds are in captive birds, zoological collections, and on isolated islands when new host–parasite associations become established. Avian malaria is particularly pathogenic in

captive penguins whenever they are exposed to mosquito vectors outside of their natural range (Stoskopf and Beier 1979; Fix et al. 1988). Well-documented cases of mortality from *Plasmodium* have not been reported in wild penguins (Jones and Shellam 1999; Sturrock and Tompkins 2007), but the introduction and spread of new mosquito vectors and the potential effects of global climate change may begin to place wild colonies at risk in future years (Miller et al. 2001; Tompkins and Gleeson 2006).

The threat that introduced avian malaria poses to endemic birds on isolated islands is substantial. The accidental introduction of *P. relictum* and the southern house mosquito (*C. quinquefasciatus*) to the Hawaiian Islands has had a devastating impact on native Hawaiian forest birds (Warner 1968; van Riper et al. 1986) and continues to play a significant role in limiting the current geographic and altitudinal distribution of remaining species (Atkinson et al. 1995; Benning et al. 2002). Of more than 70 species and subspecies of endemic forest birds present at the end of the eighteenth century, at least 23 are now extinct and 30 of the remaining species and subspecies are listed as endangered by the U.S. Fish and Wildlife Service (Jacobi and Atkinson 1995). While numerous limiting factors have contributed to these extinctions, high susceptibility to malaria is believed to be one of the most important reasons why populations of native species have collapsed at low elevations in areas where suitable habitat still exists (van Riper et al. 1986; Atkinson et al. 1995). High rates of transmission are maintained by the extremely high susceptibility of native honeycreepers (Drepanidinae) to *P. relictum* (Atkinson et al. 1995, 2000), presence of high rates of malaria transmission in the lowlands (Woodworth et al. 2005), and presence of disease-free refugia on the highest mountaintops that provide a continual source of nonimmune birds for initiating epizootics at lower elevations (Atkinson and LaPointe 2009). While many of the more rare native species are continuing to decline, at least one, Hawaii Amakihi (*Hemignathus virens*), appears to be evolving some resistance to infection, and lowland populations in some parts of Hawaii have started to rebound in recent years (Woodworth et al. 2005; Foster et al. 2007).

TREATMENT AND CONTROL

Chloroquine phosphate, primaquine phosphate, pyrimethamine–sulfadoxine combinations, and mefloquine are effective in treating canaries, penguins, and raptors with avian malaria (Remple 2004). The anticoccidial drugs sulfamonomethoxine, sulfachloropyrazine, and halofuginone are somewhat effective in treating *P. durae* in domestic turkeys and may also be effective against *P. gallinaceum*. Sulfamonomethoxine suppresses parasitemia, but does not provide full protection from mortality when given after the appearance of circulating parasites. Sulfachloropyrazine reduces mortality, but has no effect on parasitemia, suggesting that it has some efficacy against exoerythrocytic schizonts. Halofuginone delays parasitemia, but suppresses it to only a minor extent (Huchzermeyer 1993a).

While birds were some of the first experimental models for development of vaccines against *Plasmodium*, practical methods for immunizing wild birds have not been developed and this probably presents the most significant challenge to controlling infection with this approach. A variety of different experimental vaccines have been used, including use of ultraviolet light-inactivated, formalin-inactivated, and irradiated sporozoites, merozoites, and gametes, and synthetic vaccines based on parasite surface molecules (van Riper et al. 1994). Two DNA vaccines based on the circumsporozoite protein of *P. gallinaceum* and *P. relictum* have recently been evaluated in Jackass Penguins (African Black-footed Penguins, *Spheniscus demersus*; Grim et al. 2004), and canaries (McCutchan et al. 2004) exposed to natural transmission of *P. relictum* in a zoological park. Both provided protection to natural exposure to *P. relictum*, but immunity was short-lived in canaries, and birds were just as susceptible as unvaccinated controls when exposed to mosquito vectors 1 year later.

As has been demonstrated with human malaria, reductions of populations of mosquito vectors can reduce transmission of *Plasmodium*, but this method has not been widely used to control infections in wild or captive birds. Efforts to control avian malaria in Hawaiian forest birds have focused on reducing larval habitat for the introduced mosquito, *C. quinquefasciatus* (Reiter and LaPointe 2007; LaPointe et al. in press). The most cost-effective measures for captive or domestic birds include housing cage birds in screened, mosquito-proof buildings, or locating birds in areas that are isolated from wild reservoir hosts.

MANAGEMENT IMPLICATIONS

The potential risk of exposure to avian malaria should be considered when threatened or endangered species are moved outside of their normal ranges and maintained in captive propagation facilities or zoological parks where they may be introduced to new vectors and locally transmitted strains of *Plasmodium*. This risk is well documented for penguins, but should also be considered for species of birds from remote and isolated island systems that may have no prior exposure to these parasites. Similarly, the unintentional introduction of both parasites and mosquito vectors to new