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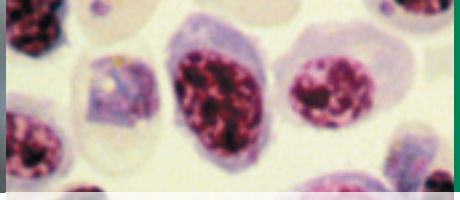

Ecology and Diagnosis of Introduced Avian Malaria in Hawaiian Forest Birds

Avian malaria is a disease caused by species of protozoan parasites (*Plasmodium*) that infect birds. Related species commonly infect reptiles, birds and mammals in tropical and temperate regions of the world. Transmitted by mosquitoes, the parasites spend part of their lives in the red blood cells of birds (Figure 1). Avian malaria is common in continental areas, but is absent from the most isolated island archipelagos where mosquitoes do not naturally occur. More than 40 different species of avian *Plasmodium* have been described, but only one, *P. relictum*, has been introduced to the Hawaiian Islands.

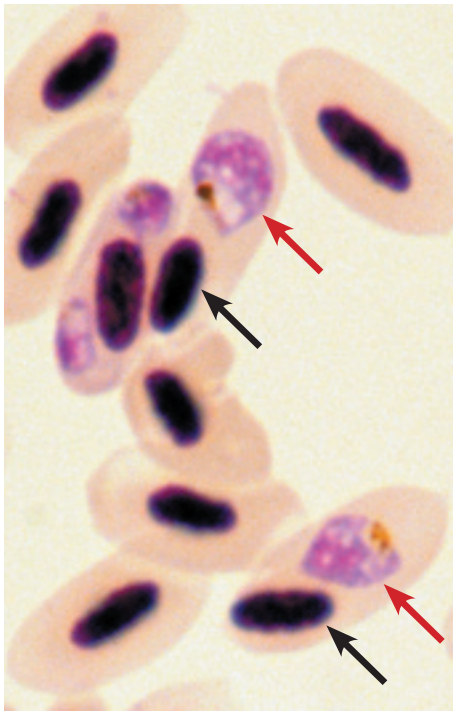

Figure 1. Blood smear from an *'apapane* infected with *Plasmodium relictum*. The parasites (red arrows) develop within the circulating red blood cells and stain purple. Red blood cell nuclei (black arrows) in parasitized cells are frequently pushed to one end of the cell.

Because they evolved without natural exposure to avian malaria, native Hawaiian honeycreepers are extremely susceptible to this disease. Malaria currently limits the geographic distribution of native species, has population level impacts on survivorship, and is limiting the recovery of threatened and endangered species of forest birds.

Altitudinal and Geographic Distribution of Avian Malaria

Several factors influence the prevalence of avian malaria across the Hawaiian archipelago. Hawai'i has a wide spectrum of climatic zones and habitats that differ in rainfall, temperature and elevation. These habitats vary in their suitability for species that harbor and transmit malaria. Mosquitoes, for example, are more likely to be found in wet, low elevation habitats with temporary or permanent bodies of standing water that provide habitat for larval development. Similarly, infection rates among birds in the islands also differ due to differences in susceptibility and how well they overlap with suitable mosquito habitat. One of the most important developments since the 1970s is the emergence of recovering lowland populations of *Hawai'i 'amakihi* (*Hemignathus virens*) in the Puna District of Hawai'i Island. These birds have changed our view of how malaria is transmitted across the larger landscape on the eastern slopes of Kīlauea Volcano

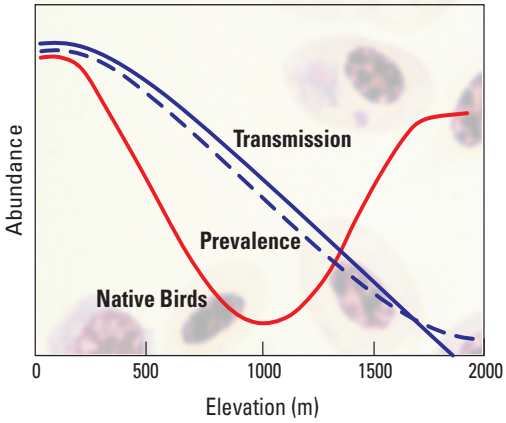

Figure 2. Our current understanding of the distribution of avian malaria on Mauna Loa and Kīlauea Volcanoes after emergence of disease-resistant, low elevation *Hawai'i 'amakihi* populations. Disease prevalence (abundance) and transmission fall as elevation increases both because of thermal limits on development of the parasite in the vector and because mosquitoes decline in numbers and become more seasonal at higher altitudes. Populations of native birds at high elevation are more diverse and include the species that have little or no natural resistance to malaria. The absence of native birds at middle elevations is still incompletely understood and may reflect lower rates of natural selection for disease resistance that occur in areas with seasonal malarial transmission. A good test of this hypothesis will be to see if these areas are recolonized over the next decade by disease resistant *'amakihi* from the lowlands.

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(Figure 2). Their presence here and elsewhere in Hawai'i indicates that some native species have the capacity to evolve resistance to this disease.

Malaria is most common at middle (900–1500 m) elevations on moist, windward sides of all of the main Hawaiian Islands where native species still occur, but has not been reported in the Northwestern Hawaiian Islands. It is uncommon to rare in alpine habitats on Mauna Loa, Mauna Kea, and Haleakalā Volcanoes and is found almost entirely in birds that have moved from lower elevations. Malaria appears to be abundant in lowland habitats in areas with recovering native bird populations, but is less common in areas that are dominated by non-native species. The disease can be found in very dry habitats, but generally only in areas where mosquito populations are supported by intermittent sources of water.

Seasonal Transmission of Avian Malaria

Malaria transmission becomes increasingly seasonal as elevation increases, both because numbers of mosquitoes are very low at higher elevations during the cooler winter months and because of thermal constraints on development of the parasite in the mosquito vector. Malaria transmission at elevations between 900 and 1500 m typically occurs during the warmest time of the year between September and December when mosquito populations reach their peak. This period follows the nesting season for most native species and the abundance of recently fledged, susceptible juvenile birds coupled with increasing mosquito populations can lead to epidemic outbreaks that may continue to the onset of colder winter temperatures in January (Figure 3). Transmission may occur throughout the year at lower elevations if suitable reservoir hosts and susceptible, uninfected birds are present.

Reservoirs of Infection

Perhaps surprisingly, the highest prevalence of malaria and the highest intensity of infections now occur in highly susceptible native species (Figure 4). Malaria may have been introduced in a songbird or game bird species to which it was closely adapted. After initial spread to native forest birds, the source host may not have become established in the islands, leaving a parasite behind that was highly pathogenic (able to inflict damage) in native birds, but with low infectivity and pathogenicity to other non-native species that became established here. Alternatively, the source host may be established in the islands, but with a range that is more restricted than the current distribution of avian malaria. We do not know the original host species, but one likely culprit is the House Sparrow (*Passer domesticus*) which maintains blood stage infections and infectiousness to mosquito vectors for long periods of time with no evidence of clinical signs. Among native species, *'apapane* (*Himatione sanguinea*) and *'amakihi* (*Hemignathus* spp.) generally have the highest prevalence of infection in the wild.

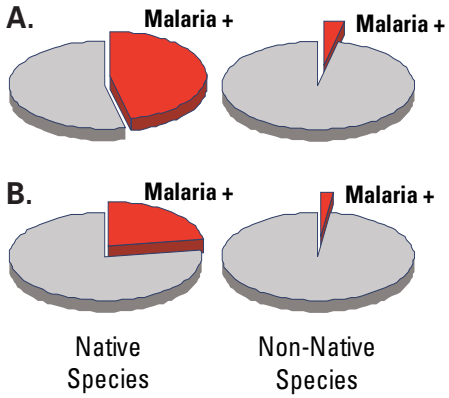


Figure 4. Prevalence of avian malaria in native and non-native species from mid-elevation habitats on the windward and leeward slopes of Mauna Loa Volcano. **A.** Kīlauea Iki Crater, Hawai'i Volcanoes National Park. **B.** Kona Unit, Hakalau Forest National Wildlife Refuge

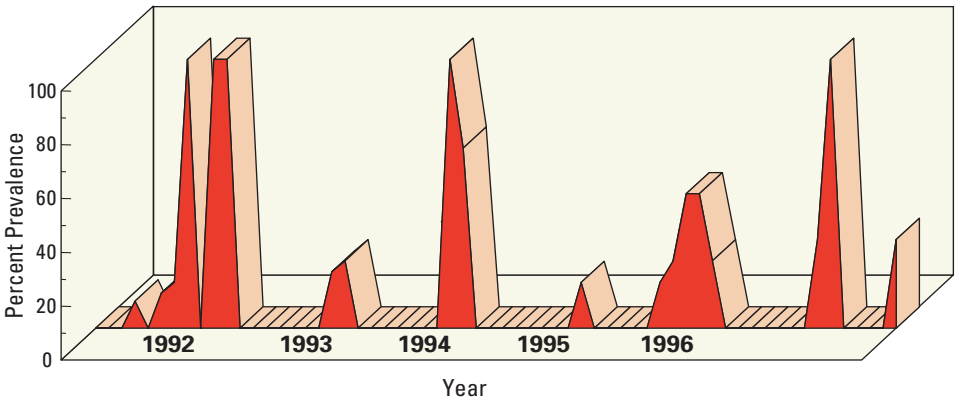


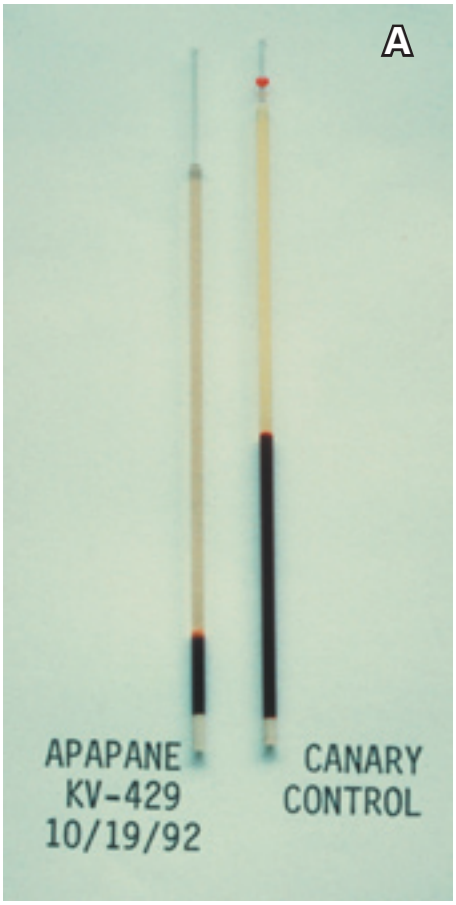
Figure 3. Percent prevalence of avian malaria in hatch-year *'apapane* at Kīlauea Iki Crater in Hawai'i Volcanoes National Park between 1992 and 1998. Epidemic outbreaks at this mid- elevation (1200 m) site are limited to the warmer months of the year between September and December. Prevalence varies from year to year for a variety of reasons including variations in rainfall, vector populations, and numbers of susceptible juvenile birds.

Mortality in Native Forest Birds

Experimental studies of the pathogenicity of avian malaria have been instrumental in documenting relative susceptibility and mortality among native and non-native species and documenting evidence of evolving resistance in some low elevation populations of *Hawai'i 'amakihi* (*Hemignathus virens*). Among species we have tested, both *'i'iwi* (*Vestiaria coccinea*) and *Maui 'alauahio* (*Paroreomyza montana*) are particularly sensitive to malaria, with mortalities of 90% and 75%, respectively, following exposure to single infective mosquito bites. Both of these species rarely occur at elevations below 1200 m, suggesting that disease transmission may be a primary factor limiting their current distribution. By contrast, *'ōma'o* (*Myadestes obscurus*) and low elevation populations of *Hawai'i 'amakihi* from the Puna District are more capable of surviving infection, with little or no mortality after exposure to single infective mosquito bites.

Signs of Acute Malaria

The earliest detectable evidence of malaria infection in highly susceptible honeycreepers is the appearance of parasites in circulating blood cells from 48 to 72 hours after exposure to an infective mosquito bite. Parasites begin rapid multiplication in the blood cells, but the first overt signs of infection do not begin until approximately seven days post-infection when declines in food consumption and activity levels first become evident. Among honeycreepers that eventually recover from infection, peak levels of parasites in the blood normally



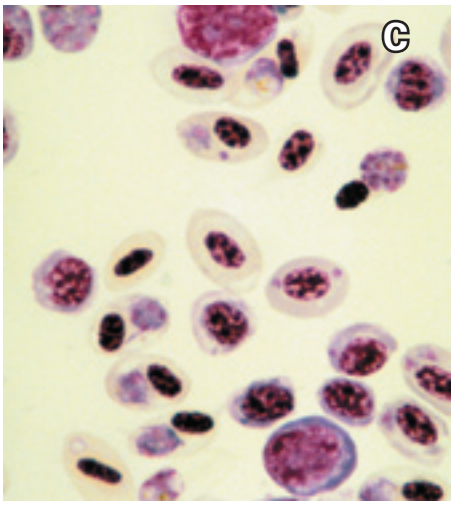


Figure 5. Characteristic signs and lesions of acute malaria. **A.** Red blood cell destruction and anemia is the primary cause of death in acute infections. After tubes of whole blood are spun in a centrifuge, volume of packed red blood cells is significantly lower in a wild *'apapane* with acute malaria (left) than in an uninfected canary. **B.** Lesions in an *i'iwi* with acute malaria. Note enlarged, blackened liver (arrow). **C.** Blood smear from an *i'iwi* with acute malaria. Mature red blood cells have been destroyed and replaced with immature red blood cells and their precursors to compensate for anemia caused by cell destruction. **D.** *Hawai'i 'amakihi* with acute malaria. At the crisis, birds become sedentary, cease feeding, and may be very susceptible to predation.

occur about 12 days post-infection when approximately 25% of the circulating red blood cells are infected. The crisis passes as the immune system begins to control numbers of parasites in the circulation. During the peak period of parasitemia or "crisis", birds become sedentary and may cease feeding altogether. Among honeycreepers that succumb to infection, up to 80% of the circulating red blood cells may be parasitized. Most deaths occur between 18 and 24 days after infection and birds are typically from 20 to 40% below normal body weight and have a prominent sternum or "keel". Gross lesions are easy to recognize at necropsy and include enlarged, chocolate brown or black liver and spleen and thin, watery blood (Figure 5). The most likely cause of death is anemia associated with destruction of red blood cells.

Chronic Malaria and Problems with Disease Diagnosis

Honeycreepers that survive acute malaria develop chronic, low level infections that may persist for the lifetime of the bird. Unlike acute infections, numbers of parasites in the peripheral circulation may be extremely low and difficult to detect by microscopy. Available experimental evidence indicates that these birds are immune to re-infection. They may exhibit no outward signs of being infected and may be in excellent body condition, yet are still infectious to mosquitoes and are excellent reservoir hosts for maintaining the disease in forest bird populations. Chronically infected birds may also carry genes for disease resistance and may be good candidates for translocation or captive propagation for restoring forest bird populations in locations where vector control is not feasible. Accurate identification of these individuals is important when assessing disease risks for an area.

Sensitivity and Specificity of Different Diagnostic Methods

Both direct and indirect methods have been developed for diagnosing malaria. Direct methods that demonstrate the parasite or a portion of its DNA sequence include microscopy and polymerase chain reaction (PCR) techniques. Microscopy is still considered the "gold standard" for malaria diagnosis because parasites are actually seen within the blood cells, but it can miss more than 70% of chronic infections because numbers of parasites are so low. PCR methods that amplify specific portions of parasite DNA are significantly

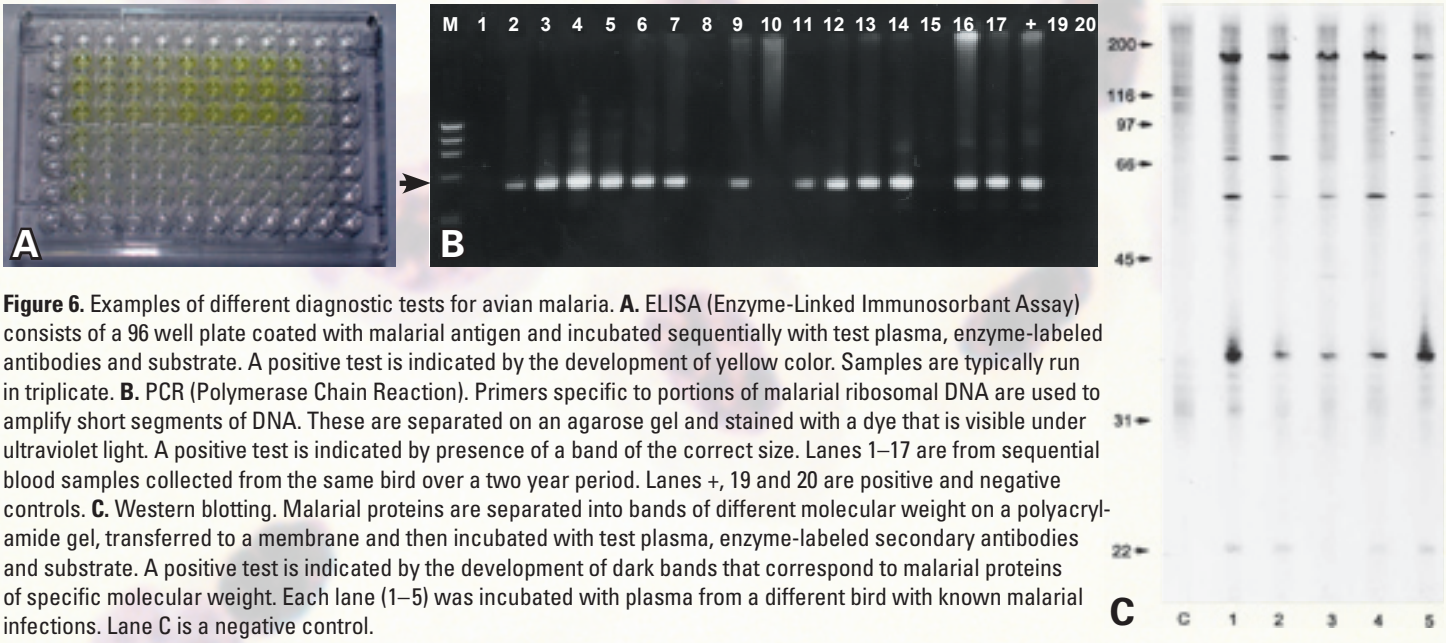


Figure 6. Examples of different diagnostic tests for avian malaria. **A.** ELISA (Enzyme-Linked Immunosorbant Assay) consists of a 96 well plate coated with malarial antigen and incubated sequentially with test plasma, enzyme-labeled antibodies and substrate. A positive test is indicated by the development of yellow color. Samples are typically run in triplicate. **B.** PCR (Polymerase Chain Reaction). Primers specific to portions of malarial ribosomal DNA are used to amplify short segments of DNA. These are separated on an agarose gel and stained with a dye that is visible under ultraviolet light. A positive test is indicated by presence of a band of the correct size. Lanes 1–17 are from sequential blood samples collected from the same bird over a two year period. Lanes +, 19 and 20 are positive and negative controls. **C.** Western blotting. Malarial proteins are separated into bands of different molecular weight on a polyacrylamide gel, transferred to a membrane and then incubated with test plasma, enzyme-labeled secondary antibodies and substrate. A positive test is indicated by the development of dark bands that correspond to malarial proteins of specific molecular weight. Each lane (1–5) was incubated with plasma from a different bird with known malarial infections. Lane C is a negative control.

more sensitive than microscopy, but are more expensive, time consuming and may require additional sequencing steps to confirm that the products originated from parasite DNA.

Indirect methods for demonstrating infection with malaria include serological (blood) screening for the presence of antibodies to the parasite. Two methods have been applied to diagnosing avian malaria in Hawai'i: Enzyme Linked Immunosorbant Assay (ELISA) and Western Blotting (Figure 6) . ELISA is more sensitive than Western Blotting, but may require additional tests by Western Blotting to verify positive results. Western Blotting is the most specific serological test available for avian malaria and can be used to verify both ELISA and PCR tests.

Microscopy, serology and PCR can all play important roles in providing accurate diagnostic information about malarial infections in Hawaiian birds. When used alone, the tests vary in their ability to accurately detect chronic malarial infections with low intensities. Microscopy is most likely to miss chronic infections, but it is extremely accurate when parasites are detected and can be used to obtain information on intensity of infection and host cellular responses. Serological methods are extremely sensitive for detecting older infections in recovered birds because persistent infections stimulate antibody production and cellular immunity to the parasite. They provide no information about parasite intensity or morphology, however, and are also likely to miss very early acute infections, before the host is able to produce antibodies to the parasite.

Available experimental evidence indicates that PCR is not quite as sensitive as serological methods for detecting chronic infections, but it is much more sensitive than microscopy. When used in combination, these diagnostic methods can complement each other and provide critical information to resource managers about the prevalence and distribution of avian malaria in habitats that are being considered for restoration of threatened and endangered forest birds.

Recommended Reading

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