4. Avian malaria in Europe: an emerging infectious disease?

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Abstract

Emerging infectious diseases have had, and likely will have, a great impact on human and animal populations, and on biodiversity. Avian malaria is one such disease, which reached epidemic levels soon after its introduction in Hawaii and contributed to a massive decrease of avian biodiversity on the island group. Avian malaria is also a common infection amongst endemic and migratory birds in Europe, but appears to cause little harm among the native birds. However, frequent deaths of non-indigenous birds due to avian malaria are being reported. In this chapter, we consider whether avian malaria can reach epidemic proportions in Europe as an emerging infectious disease and as a result of climate change. In an ecological field study, we investigated the occurrence of avian malaria in Rotterdam zoo in the Netherlands during the summer of 2003. In a longitudinal approach mosquito dynamics were studied and a cross-sectional study was undertaken to estimate parasite prevalence in exotic birds kept in the zoo. Adult mosquitoes were caught throughout the study period and were examined for parasite infections. Of the 575 adult mosquitoes caught in traps, 61 percent was *Culex pipiens pipiens*. Six mosquitoes of this species were infected with malaria. Nine birds, belonging to eight different species, were infected with malaria, out of 81 birds that were examined. Twelve other birds died later that summer and were diagnosed with avian malaria, most of them being black-footed penguins. The peak in mortality occurred in the third week of August, two weeks after the peak in larval mosquito densities. It appeared that there is annual seasonal malaria transmission within Rotterdam zoo. We suggest that these infections result from a reservoir of parasites living within less susceptible exotic birds that are kept in the zoo and in endemic birds living within or near the zoo. These field data support our view that an outbreak of avian malaria among the endemic bird population of Europe is unlikely to happen under the current circumstances. Avian malaria is a common parasitic infection in the endemic and migratory bird populations, and after a long history of co-evolution, the parasites are causing what appears to be little harm. However, a change of climate or parasite mutational events could alter the equilibrium between parasite and host, potentially resulting in an epidemic. Frequent screening of the endemic and migratory bird populations should therefore be employed to prevent an outbreak such as happened in Hawaii.

Keywords: avian malaria, *Plasmodium*, bird, mosquito, Europe, emerging infectious disease

Introduction

Among vertebrates, infectious diseases can have a devastating effect on populations. Through co-evolution, most animals have developed an effective immune response to infections with pathogens and parasites[1] and they can therefore survive periodic outbreaks of diseases. Some infections express a greater virulence than others, depending on the frequency and intensity of parasite-host interactions as well as the genetic make-up of parasite and host (Daszak *et al.* 2000). Geographic isolation may sometimes prevent the exposure of animal populations to parasites, rendering such populations highly susceptible to introduced pathogens and parasites. Historic examples of such events are the accidental importation of plague and smallpox into Europe

[1] In this paper pathogens include viruses, fungi, protons and bacteria, and parasites include protozoa and nematodes.

(Harrison 2004) and measles into South America (Black *et al.* 1974), resulting in high mortality in the affected population.

Here we discuss avian malaria, a parasitic disease affecting birds. As with human malaria parasites, avian malaria parasites are transmitted from one vertebrate to another through mosquito bites (Valkiunas 2005). Although in human malaria only mosquitoes of the genus *Anopheles* transmit the parasites, avian malaria parasites are usually transmitted by other mosquito genera, of which *Culex* spp. are the most recognised vectors. We discuss the possibility of avian malaria becoming an infectious disease in Europe, whereby we use a recent outbreak of avian malaria in Hawaii as an example. Moreover, we show novel data from a recent study of avian malaria in the Rotterdam zoo. Could the occurrence of an avian malaria parasite reservoir and abundant vector populations result in epidemic levels of avian malaria in Europe?

An emerging disease is defined as 'an infection that newly appears in a population, or has existed but is (rapidly) increasing in incidence or geographic range' (Lederberg *et al.* 1992). There are many examples of infectious diseases, such as the plague that swept through Europe and Asia, killing millions of people during the Middle Ages (Harrison 2004), or 'Spanish influenza', killing an approximated 20 million people across the globe between 1918-1919 (Taubenberger *et al.* 1997). More recently, the vector-borne Chikungunya virus has rapidly spread across the islands of the Indian Ocean, with a peak impact on the island of Réunion, where almost one third of the human population was affected by the disease (Schuffenecker *et al.* 2006). Another example is the arbo-virus bluetongue, which showed up Italy in 1998, affecting millions of sheep (Baylis *et al.* 2001). Bluetongue continues to plague Europe with a new outbreak occurring in north-western Europe in 2006 (see Chapter 7), affecting sheep and cattle and causing huge economic losses (Mehlhorn *et al.* 2007). A final and well-known example is the West Nile virus, which was accidentally introduced in the United States in 1999 and spread rapidly across the North-American continent with numerous casualties and many hospitalisations (Tyler 2004). All these emerging diseases follow a common path where a highly virulent pathogen enters and rapidly spreads in a susceptible population whereby it kills a relatively high percentage of its hosts.

Avian malaria: the parasites

The term malaria has confusingly been used for either all *Haemosporidians* (class *Sporozoa*, order *Haemosporida*), a group of protozoans that use blood-sucking dipteran insects as vectors to complete their life-cycle, or strictly parasites of the genus *Plasmodium*. Although haemosporidian parasites are genetically closely related, the life-cycle, vector species, and epidemiology of parasites from different families are very different (Valkiunas *et al.* 2005). Therefore, in this chapter, when we refer to avian malaria we mean parasites of the genus *Plasmodium*.

Thirty-eight morphologically different avian *Plasmodium* species have been described. The parasite species that has received the most attention is *P. relictum*. This parasite is not only well known from avian malaria in Hawaii (Van Riper III *et al.* 1986) and avian malaria outbreaks in zoos all around the world (Cranfield *et al.* 1994, Fix *et al.* 1988, Graczyk *et al.* 1995), but it has also been used as a model species to study human malaria during the end of the 19th and beginning of the 20th century (Garnham 1966).

Unfortunately, there is little information available on the vector species of avian malaria. However, since much experimental work has been carried out with *P. relictum*, it is known that this parasite

can complete its life cycle in 26 different species of the Culicidae family, including the genera *Aedes*, *Anopheles*, *Culex* and *Culiseta* (LaPointe *et al.* 2005, Valkiunas 2005). The life cycles of the different species of avian malaria parasites are generally similar. Figure 1 shows a schematic overview of the life cycle of avian malaria, using *P. relictum* as an example.

The sexual reproduction phase of the parasite takes place in the mosquito midgut, where male and female gametes fuse to form a zygote, which in turn, differentiates into the mobile ookinete. The ookinete penetrates the midgut to form an oocyst on the outer midgut wall of the mosquito. Within the oocyst, hundreds of sporozoites are being produced, until the oocyst eventually ruptures, resulting in a release of the sporozoites. These migrate to the salivary glands of the mosquito, after which the mosquito is able to transmit the sporozoites while blood-feeding on a bird. The time for the parasite to develop within the mosquito takes approximately seven days.

When blood-feeding on a bird, the mosquito injects its saliva, containing various enzymes to enhance blood uptake and prevent clotting, together with the sporozoites, which end up in

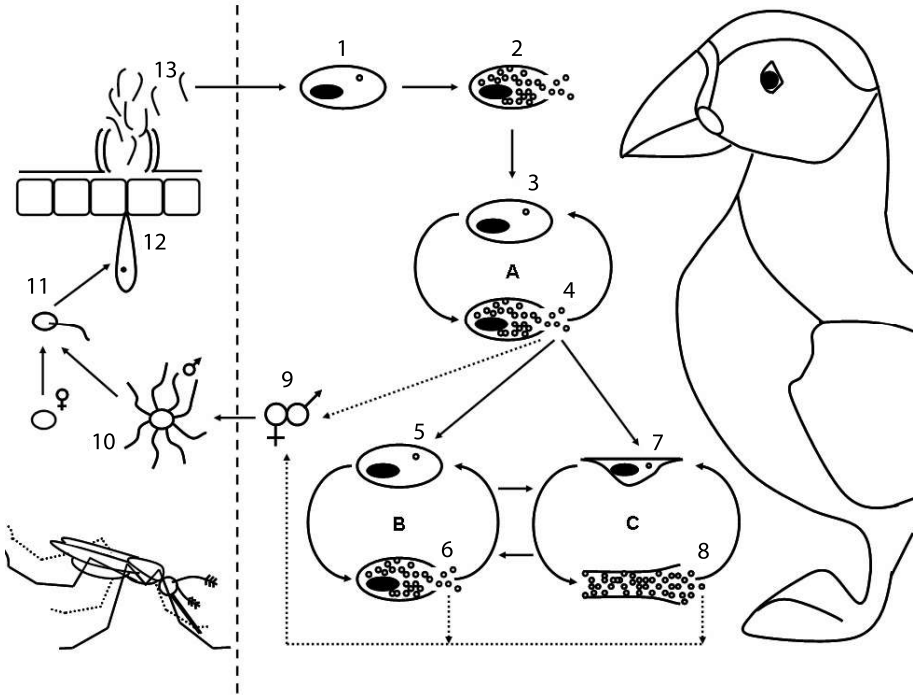

Figure 1. Life cycle of the avian malaria parasite Plasmodium relictum. On the right the asexual life cycle in the bird is depicted: 1) infected reticuloendothelial cell by sporozoites that are injected in the blood stream by the mosquito; 2) emerging cryptozoites; 3) infected macrophage; 4) emerging metacryptozoites; 5) infected erythrocyte; 6) emerging erythrocytic merozoites; 7) infected endothelial cell of the capillaries; 8) emerging phanerozoites; 9) male and female gametocytes. A) Primary exoerythrocytic cycle; B) Erythrocytic cycle; C) Secondary exoerythrocytic cycle. On the left the sexual life cycle in the mosquito is illustrated: 10) male and female gametes; 11) fertilised zygote; 12) ookinete moving through midgut wall, and 13) emerging sporozoites from oocyst.

the bird's blood stream. The sporozoites invade the reticular cells of various organs, such as the spleen, and tissues, such as skin tissue, where they develop into cryptozoites. The merozoites that are developing within the cryptozoites are, unlike the merozoites in human malaria, unable to infect red blood cells immediately, but undergo a second exo-erythrocytic cycle, during which they invade the macrophages in many organs. Part of the resulting metacryptozoites stay in the primary exo-erythrocytic cycle and infect new macrophages again. The remaining parasites either (a) enter the erythrocytic cycle, where they invade the red blood cells and multiply to a schizont; (b) invade a red blood cell to immediately develop into a gametocyte; (c) enter the secondary exo-erythrocytic cycle, where they invade the endothelial cells of many organs (including the brain and liver) in which the phanerozoites develop. A proportion of the merozoites from the erythrocytic cycle stays in the erythrocytic cycle and reinfects new erythrocytes. The remaining of the parasites can either infect a new red blood cell to develop into a gametocyte or enter the secondary exo-erythrocytic cycle. The phanerozoites of the secondary exo-erythrocytic cycle can also develop into gametocytes or enter the erythrocytic cycle or reinfect the endothelial cells, where they can stay for the remainder of the host's life. Periodic relapses, resulting from these dormant phanerozoites, often occur and are for instance related to a weakened immune system and environmental stress and often synchronised to the breeding season (Valkiunas 2005). The time to maturation of the first generation of metacryptozoites, the prepatent period, is usually less than five days for *P. relictum*.

It is believed that avian malaria parasites in wild birds are relatively harmless, whereas morbidity in captive birds can be severe, frequently leading to death. However, observations from the field may be skewed since the observed birds are often captured with mist nets which will result in relatively more healthy than sick birds being caught (Van Riper III *et al.* 1986, Westerdahl *et al.* 2005).

Ecological field study: avian malaria in Rotterdam zoo

Background

During the summer of 2003 we conducted an ecological field study in Rotterdam zoo, hereafter referred to as the zoo. The zoo has experienced a history of problems with avian malaria and frequent deaths occurred in their black-footed penguin (*Spheniscus demersus*), common puffin (*Fratercula arctica*) and common guillemot (*Uria aalge aalge*) populations. Usually, the birds were given prophylactic treatment with chloroquine and primaquine to control the disease during peak transmission season, which is not a desirable long-term solution. Therefore, a study was conducted to examine the prevalence of avian malaria among the exotic birds kept in the zoo and the mosquito dynamics within the zoo and its surroundings in order to understand the dynamics of the disease and its vectors.

Material and methods

The mosquito population was monitored from May to September 2003 within the zoo and its close surroundings. Twenty-four potential larval breeding sites were selected at the beginning of the study and were visited twice a week throughout the study period. Larvae were sampled randomly using a standard white dipper, as described in Service (1993). The number of dips that were taken depended on larval densities: 25 dips were taken in sites with a high larval abundance (>2.5 larvae per dip), 50 dips in sites with intermediate larval abundance (between 1 and 2.5 larvae per dip)

and 100 dips in sites with low larval abundance (less than one larva per dip). Larvae were collected and morphologically identified.

The adult mosquito population was monitored with three mosquito traps (Mosquito Magnet®, American Biophysics Corporation, North Kingstown, RI, USA). One trap was placed in the residence of the puffins and guillemots, another trap was placed next to a large water source in the surroundings of the penguin enclosure, and the last was placed at a central location in the zoo (Figure 2). The traps were emptied twice a week and mosquitoes were stored on silica gel. In the laboratory the collected mosquitoes were morphologically identified (Van Haren and Verdonschot 1995) and counted. Later, all mosquitoes were checked for infections with avian malaria using a Polymerase Chain Reaction (PCR) as described by Waldenström *et al.* (2004), which identifies both *Plasmodium* spp. and *Haematoproteus* spp. As *Haematoproteus* spp. are not transmitted by mosquitoes, positive samples indicated *Plasmodium* spp. infections. DNA isolation was performed with a standard phenol extraction and ethanol precipitation (Snounou *et al.* 1993a).

A cross-sectional parasitological study was performed during 4-5 July 2003. Approximately ten percent of each bird population, representing 44 species, was randomly selected and from each bird 200 µl of blood was drawn, which was immediately mixed with EDTA. On 5 August, blood was taken from all black-footed penguins in the same way. DNA was extracted with the QIAamp DNA mini kit, with the adjustment that the blood was incubated with 200 µl lysis buffer and 20 µl proteinase K at 55 °C overnight, due to clotting of the blood. A general PCR to identify *Plasmodium* spp. was used on these blood samples, which detects all *Plasmodium* species, including human malaria (Snounou *et al.* 1993b).

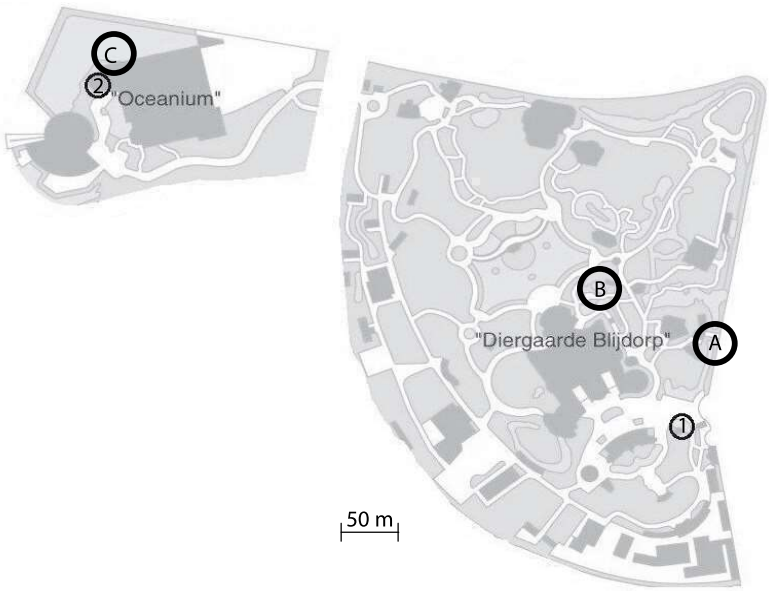

Figure 2. Map of Rotterdam zoo. Number 1 indicates the residence of the black-footed penguins, number 2 the residence of the puffins, guillemots and blacklegged kittiwakes. A, B and C indicate the location of the mosquito traps. The zoo is divided in two parts by railway tracks.

Results

Regarding the larval sampling, the most abundant mosquito species in the zoo was *Culex pipiens pipiens* (hereafter referred to as *Cx. pipiens*). Other frequently caught mosquito larvae in the zoo were *Culiseta annulata* and mosquitoes of the *Anopheles maculipennis* complex. Considering the adult catches, a total number of 575 mosquitoes were caught in the traps (Table 1). The most abundant species in the traps were again *Cx. pipiens* (60.5%), *Culiseta annulata* (20.5%) and *Anopheles maculipennis* spp. (8.5%). The mosquito traps experienced temporary malfunctioning, with all traps not operating during week 34, which caused a dip in the mosquito density measured during that week. Moreover, mosquito storage problems resulted in a decay of some mosquitoes beyond recognition and a loss of some of the collections. Therefore, it was not possible to follow adult mosquito dynamics accurately through time. The mosquitoes of the *An. maculipennis* complex were not identified to species by PCR, but it is assumed these mosquitoes were of the *An. messeae* species, which is the most abundant species of this complex in The Netherlands (Takken *et al.* 2002).

As for actual mortality of birds due to malaria, the zoo experienced 12 cases during the study period, between 20 July and 17 August: six black-footed penguins, three common guillemots, one black-legged kittiwake (*Rissa tridacttyla pollicaris*), one European eider (*Somoateria mollissima*) and one demoiselle crane (*Anthropoides virgo*). These were all diagnosed with avian malaria by the veterinary surgeon using microscopy. The peak in mortality due to avian malaria occurred in week 33 (11 August). The peak in larval abundance of *Cx. pipiens* occurred two weeks earlier (in week 31, 28 July; Figure 3). The adult *Culex pipiens* population also reached its highest numbers between week 31 and week 33.

In the cross-sectional study, blood was taken from 81 individual birds, representing 42 different species. Nine birds, representing eight species, were found PCR-positive for *Plasmodium* spp. infections (Table 2). None of the infected birds showed any symptoms of a malaria infection. Six black-footed penguins were enrolled in this study, none of them were infected. At the next screening of the entire black-footed penguin colony a month later during peak transmission season (first week of August), eight out of the 28 birds were infected with *Plasmodium* spp.

Table 1. Cumulative number adult mosquito species that were caught in Rotterdam zoo in three mosquito traps between May and September 2003.

Mosquito species	Number
Culex pipiens	348
Culiseta annulata	118
Anopheles maculipennis	49
Coquillettidia richiardii	6
Culex modestus	3
Anopheles algeriensis	3
Culiseta suborchea	1
Culex terrestans	1
Unknown	46
Total	575

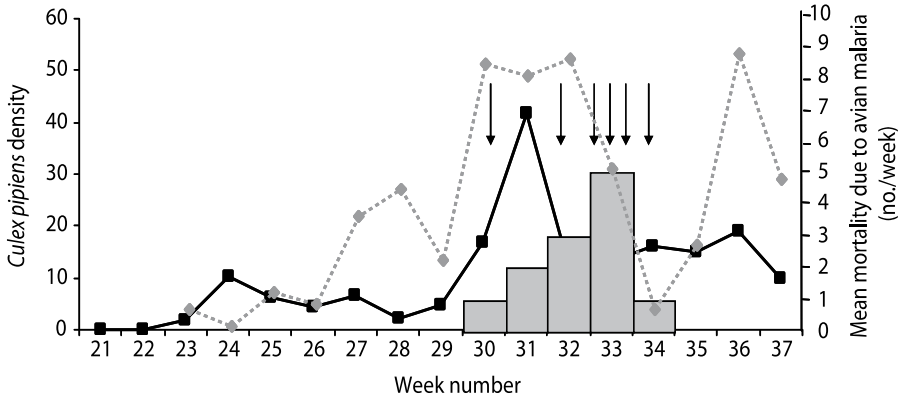

Figure 3. Larval (black line; number of larvae per dip) and adult (gray dotted line; cumulative number of adults) densities of Culex pipiens*. Larvae were sampled weekly from 24 selected larval habitats and adults were collected from three mosquito traps, both between May and September 2003 in Rotterdam zoo. The traps were malfunctioning in week 34. The grey bars indicate the numbers of birds in captivity that died per week due to avian malaria. The arrows indicate when infected mosquitoes were collected.*

Table 2. Bird species of which blood was examined during a cross sectional study on 4 and 5 July 2003 in Rotterdam zoo. Numbers of individuals that were infected with avian malaria are given.

Bird species English name *Scientific name*	Number of birds examined	Infected with *Plasmodium* spp.
Andean guan *Penelope montagnii montagnii*	1	
Bahama pintail *Anas bahamensis bahamensis*	1	
Black stork *Ciconia nigra*	1	
Black-footed penguin *Spheniscus demersus*	6	
Black-legged kittiwake *Rissa tridacttyla pollicaris*	5	
Blue-eyed cockatoo *Cacatua ophthalmica*	1	
Blue-winged kookaburra *Dacelo leachii*	1	
Caribbean flamingo *Phoenicopterus ruber rubber*	3	
Common goldeneye *Bucephala clangula*	1	
Common guillemot *Uria aalge aalge*	2	1
Common puffin *Fratercula arctica*	2	
Common shelduck *Tadorna tadorna*	3	1
Common shoveler *Anas clypeata*	1	
Dalmatian pelican *Pelecanus crispus*	2	
Demoiselle crane *Anthropoides virgo*	1	
Emu *Dromaius novaehollandiae*	2	
Eurasian eagle owl *Bubo bubo bubo*	1	
European eider *Somateria mollissima mollissima*	1	
European goosander *Mergus merganser merganser*	3	
Falcated duck *Anas falcata*	3	
Fulvous whistling duck *Dendrocygna bicolor*	4	1

Table 2. Continued.

Bird species English name *Scientific name*	Number of birds examined	Infected with *Plasmodium* spp.
Glossy ibis *Plegadis falcinellus*	1	1
Greater flamingo *Phoenicopterus roseus*	7	2
Helmeted guineafowl *Numida meleagris*	1	1
Long-billed corella *Cacatua tenuirostris tenuirostris*	1	
Mallard *Anas platyrhnchos*	1	
Manchurian crane *Grus japonensis*	2	1
Mandarin Duck *Aix galericulata*	3	
Marabou stork *Leptoptilos crumeniferus*	1	
Mexican military macaw *Ara miliaris mexicana*	1	
Mitchell's cockatoo *Cacatua leadbeateri*	1	
Ostrich *Struthio camelus*	1	
Palawan pacock pheasant *Polyplectron emphanum*	1	
Red junglefowl *Gallus gallus*	2	
Red-billed curassow *Crax blumenbachii*	1	
Red-crested pochard *Netta rufina*	2	
Scheepmaker's crowned pigeon *Goura scheepmakeri sclaterii*	1	
Smew *Mergus albellus*	4	
Snowy owl *Nyctea scandiaca*	1	1
South African crowned crane *Balearica regulorum regulorum*	1	
White-naped crane *Grus vipio*	2	
White-winged wood duck *Cairina scutulata*	1	
Total	81	9

parasites in their blood. Six days after sampling, one positive juvenile bird died with symptoms of avian malaria. The other seven birds, all adults, survived the summer. However, all black-footed penguins, puffins and guillemots were given prophylactic treatment the third week of August and were taken into quarantine, which probably prevented more fatalities.

Of all adult *Cx. pipiens, Culiseta annulata* and *Anopheles maculipennis* s.l. collected in the traps, only *Cx. pipiens* mosquitoes were infected with avian malaria parasites. Six specimens were found positive with *Plasmodium* (1.7%). These were all captured between 22 July and 19 August, which coincided with the peak in bird mortality (Figure 3). The infected mosquitoes were spread randomly throughout the zoo, as infected mosquitoes were found in each of the three traps.

Discussion

This field study shows the various components of an entire avian malaria cycle. Six infected *Cx. pipiens* were found (1.7 percent of all *Cx. pipiens* adults caught), whereas none of the other mosquito species were found to be infected. However, *Culiseta annulata* has been identified as a competent vector of *P. relictum* (Valkiunas 2005) and an infection within this species could have been missed due to the low overall infection rate that was observed. Overall, our findings suggest that *Cx. pipiens* was the main vector of avian malaria in the Rotterdam zoo. This species was also

found to be an avian malaria vector in the Baltimore zoo, USA, together with *Culex restuans* (Beier and Stoskopf 1980). *Culex pipiens* was the dominant species in the zoo, probably due to many water bodies, such as water ponds, puddles and buckets within the zoo and water storage containers in the public vegetable gardens just outside the zoo. These are ideal breeding habitats for *Culex* spp., which frequently breed in artificial collections of water, whereas *Anopheles* spp. and *Culiseta* spp. more often breed in natural water bodies.

Unfortunately, due to some unforeseen storage problems and malfunctioning of the mosquito traps, there are some gaps in the data of the adult mosquito collections. Therefore, the larval density was used to compare vector and disease dynamics. The immature stages of *Cx. pipiens* reached their peak density at the end of July, just two weeks before the peak of avian mortality. The development of the parasite within the mosquito is dependent on weather conditions, but takes at least seven days during the summer. The incubation time for *P. relictum* from sporozoites to blood stages with clinical symptoms takes up to five days (Valkiunas 2005). Therefore, it is very likely that the sudden increase in numbers of adults of *Cx. pipiens* was a direct cause for the peak mortality observed two weeks later.

Eleven percent of the exotic birds in Rotterdam zoo were infected with *Plasmodium* spp. infections, which demonstrate that such infections may be quite common. These were probably chronic infections since all blood smears of the birds that appeared to be infected using PCR remained below detectable level using microscopy, except for the snowy owl (*Nyctea scandiaca*), who had a very low parasitaemia. Also, none of the infected birds showed symptoms of infection and this survey was done one month before peak transmission season. Apart from the common guillemot, there are no records of avian malaria within these infected species. Wild lesser flamingos (*Phoeniconaias minor*) have been reported with *P. relictum* (Peirce 2005). Unfortunately, we were not able to identify the avian malaria parasites from both infected birds and mosquitoes to species level.

Chronically infected birds living within the zoo perimeters can have important implications for the spread of avian malaria, since they can act as a reservoir. However, another possibility is that the source of infection is coming from infected endemic local birds. Unfortunately, there are no data available on the prevalence of the *Plasmodium* spp. parasites in endemic wild birds, resident in the zoo or in the Netherlands. In Baltimore zoo, the source of avian malaria infection among their black-footed penguin colony resulted from wild passerine birds living in the zoo (Cranfield *et al.* 1990). In Europe, it is well known that many bird species, especially passerines, harbour avian malaria parasites in their blood (Scheuerlein and Ricklefs 2004). *Plasmodium relictum*, most probably the causative agent for avian malaria in the zoo, has been reported in many different wild bird species, for instance in Spain, Bulgaria and Czech Republic (Blanco *et al.* 1997, Figuerola *et al.* 1999, Shurulinkov and Golemansky 2003, Votypka *et al.* 2003). It is therefore highly likely that the wild birds living in the zoo are also infected with avian malaria parasites. Therefore, the source of avian malaria infections could result from both the exotic birds kept in the zoo and the endemic wild birds living in its surroundings. It is possible that the parasite is relatively harmless in their reservoir hosts, and that only certain bird species are highly susceptible.

Migratory birds passing through The Netherlands could be another source of avian malaria. It is known that migratory birds carry avian malaria parasites (Perez-Tris and Bensch 2005, Shurulinkov and Golemansky 2003, Westerdahl *et al.* 2005). However, migratory birds pass by in the spring and autumn, whereas the peak in vector abundance and mortality caused by avian malaria occurs in the summer (see above). Moreover, studies on two migratory birds, the ruff (*Philomachus pugnax*)

and little stint (*Calidris minuta*), which migrate from Africa to the high northern latitudes, have shown that these passerines can be heavily infected with malaria parasites in their African habitat. However, neither of these species were found infected when travelling through The Netherlands or breeding in the Arctics (Mendes *et al.* 2005). The authors proposed three possible explanations for this phenomenon. First, only the healthy birds start their journey. This is supported by a recent study which simulated migration flight in a wind tunnel with red knots (*Calidris canutus*); birds with a lower immunity level refused to fly (Hasselquist *et al.* 2007). Second, infected birds that do start the flight have a lower chance of survival due to the infection. However, the wind tunnel experiments revealed that a long distance flight did not have an effect on the immunity levels of the red knots (Hasselquist *et al.* 2007). Third, the immune system may have suppressed the infection before the birds reach the northern latitudes. This seems plausible, since the immune system is not as suppressed by the long flight, as has always been assumed (Hasselquist *et al.* 2007). With recent molecular techniques to study parasite lineages, it appears that 'hitchhiking' of parasites is not as common as previously thought (Hellgren *et al.* 2007). This study distinguished between parasite lineages on sub-morphological level of migratory and endemic birds in Africa and Europe, and showed that only one of the 65 encountered *Plasmodium* lineages were present in both Africa and Europe, meaning that the parasites from the migratory birds are hardly ever transmitted to European birds at stop-over or breeding sites. Apparently, parasites that hitchhike from Africa to Europe appear not to leave their ride.

In summary, it seems unlikely that the migratory birds are the cause for the annual outbreaks of avian malaria in zoos. Our results suggest that there is an indigenous reservoir of avian malaria parasites within the exotic and endemic birds that reside near or within the zoo perimeters.

Avian malaria: an emerging disease in Hawaii

In the beginning of the 20th century, avian malaria spread rapidly over the islands of Hawaii, contributing to the extinction of many endemic bird species. Avian malaria parasites were likely to have been present in migratory birds that visit Hawaii annually. However, without a suitable mosquito vector present on the islands, the parasites never came in contact with the endemic bird population. This all changed when a ship named 'Wellington' brought water from the west coast of Mexico to the island of Maui in 1826 and accidentally introduced the tropical house mosquito *Culex quinquefasciatus* as a new species in the local ecosystem (Warner 1968). Avian malaria did not reach epidemic levels in Hawaii before the turn of the 20th century. It is believed that by then the mosquito population was fully established and that the outbreak of avian malaria was initiated by the introduction of over 200 exotic birds in the early 1900s, any number of which may have served as a reservoir of the malaria parasite *P. relictum* (Van Riper III *et al.* 1986, Warner, 1968). During the last century, 23 of the 71 endemic bird species believed to inhabit the islands at the end of the 18th century, became extinct and another 30 are currently listed endangered (Atkinson *et al.* 1995). The emergence of avian malaria on Hawaii is believed to play a key role in these extinctions.

Key factors in the success of the avian malaria parasites in Hawaii are time and isolation. Hawaiian's avian population has evolved in almost near-isolation over a period of approximately 70 million years (Mac *et al.* 1998). The first Polynesian settlers arrived about a thousand years ago and the first Europeans arrived as recently as 1778. The Hawaiian bird population, being isolated for that long, did not have a history of co-evolution with avian malaria parasites and therefore the relatively benign parasites in the introduced exotic birds were highly virulent when infecting the naïve Hawaiian birds.

Avian malaria in Europe

Various studies on avian malaria parasites across Europe show that avian malaria parasites are common in endemic bird populations. *Plasmodium* spp. infections have been reported in at least 36 passerine European bird species, spread around Europe (reviewed in Scheuerlein and Ricklefs 2004), as well as in migratory birds passing through Europe (Perez-Tris and Bensch 2005, Shurulinkov and Golemansky 2003, Westerdahl *et al.* 2005). However, unlike Hawaii, there are no reports of extinction or high mortality of bird species as a result of an infection with avian malaria in Europe.

The vector, parasites and hosts of avian malaria have co-existed since ancient times in Europe and have thus co-evolved together. Therefore, an arms race between both the host and the parasite has been running since the first contact between parasite and host. Parasites will try to increase their transmission potential by being more infectious within their host, whereas the host will respond by trying to reduce the parasite exploitation (Ewald 1995). When such co-evolved parasites subsequently invade a naïve individual, they can be extremely virulent due to the lack of an evolved immune response of the new host to this parasite (Mackinnon and Read 2004). This is exactly what happened when avian malaria parasites were introduced in the Hawaiian bird population; the otherwise relatively benign parasites to the introduced exotic birds became extremely virulent in the naïve Hawaiian birds. Natural selection, however, quickly favours those hosts with some degree of immunity. This is already happening in Hawaii, where some of the endemic Hawaiian bird species are gaining resistance against the malaria parasites (Woodworth *et al.* 2005) and behavioural adaptations such as daily migration to the mosquito-free highlands to spend the night and the covering of vulnerable areas by plumage to reduce mosquito bites are examples of adaptations of the endemic bird population to the avian malaria parasite (Valkiunas 2005, Van Riper III *et al.* 1986).

The impact of avian malaria on endemic avian wildlife in Europe will thus be less. However, some exotic birds that are kept in European zoos are facing greater problems with the disease. These birds originate from areas where there is either no transmission or transmission of other malaria species and may come into contact with the local avian malaria parasites. There are many reports of avian malaria in zoos in Europe and the USA (Cranfield *et al.* 1994, 1990, Fix *et al.* 1988, Graczyk *et al.* 1995). The causative agents that are reported are *P. relictum* and *P. elongatum* (Cranfield *et al.* 1994). Mainly individuals of the black-footed penguins are infected with malaria, which often results in seasonal deaths. There are also lethal cases known among puffins (Loupal and Kutzer 1996), common guillemots, European eiders and kittiwakes (W. Schaftenaar, unpublished data). Many zoos have decided to give their penguin population *Plasmodium* prophylaxis during peak transmission season. These medications can have adverse effects on the birds, resulting in neurological dysfunction at high and repetitive dosages (Wunschmann *et al.* 2006).

We conducted a questionnaire survey on the prevalence of avian malaria between 1995 and 2003 in eight zoos in The Netherlands and Belgium. All of these zoos were asked for cases of malaria among their avian population during this time period. The survey revealed that all participating zoos experienced mortality due to avian malaria in their birds, predominantly in their penguin populations. Most cases occurred in August and September (Figure 4), though deaths occurred all year-round.

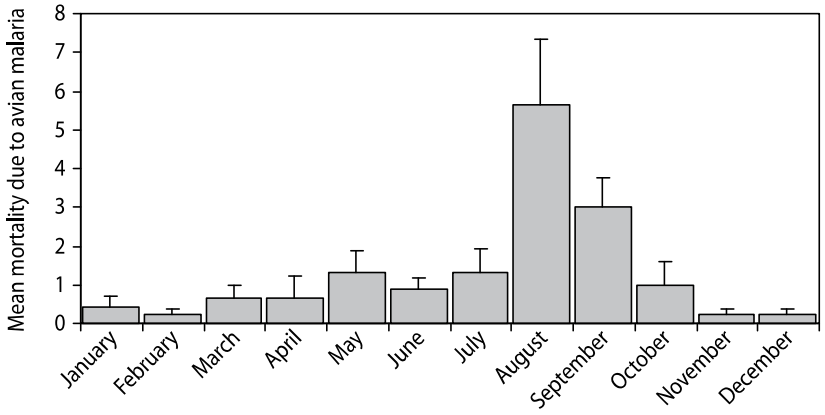

Figure 4. Monthly reported mortality caused by avian malaria (mean ± standard error) in bird populations in eight zoos in the Netherlands or Belgium from 1995 to 2003.

The juvenile birds were at highest risk: 41 percent of all observed deaths were among birds less than one year old. This is probably due to a lower immunity of these young birds (Lozano and Lank 2003). These findings are, however, in contrast with the infection percentages found in other studies, where the majority of infections were among adult birds (Mendes *et al.* 2005, Merila and Andersson 1999, Sol *et al.* 2000, Valkiunas 2005). However, an increased intensity of infection was reported among the juveniles compared to the adults, even though the prevalence was lower (Sol *et al.* 2000), which can explain an increased mortality observed in this age group. Remarkably, the annual cumulative number of cases of avian malaria reported in zoos increased over time (Linear regression, $p < 0.001$, Figure 5). Unfortunately, we do not have data on the growth of the bird population of each zoo during this time period. Therefore, the observed increase of reported avian malaria cases could be a reflection of a growing avian population. Also, an increased awareness towards an avian malaria diagnosis may have resulted in an increased number of reported avian

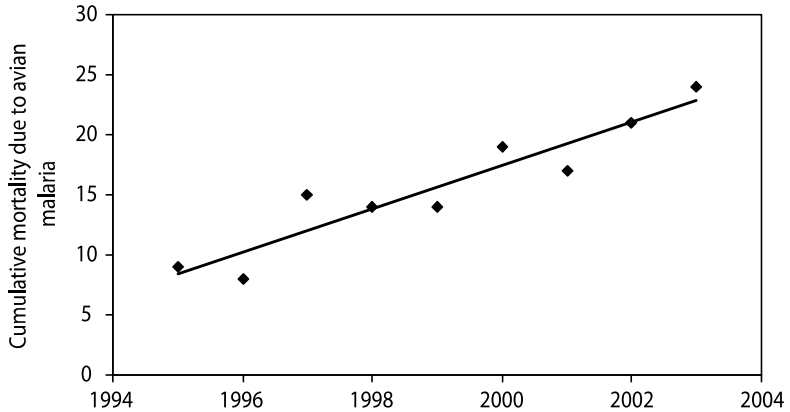

Figure 5. Annual cumulative mortality due to avian malaria in bird populations in eight zoos in the Netherlands or Belgium from 1995 to 2003.

malaria cases. However, it is also highly plausible that there was an increase in transmission rate of avian malaria between 1995 and 2003, which suggests that avian malaria is an emerging infectious disease amongst the avifauna in Dutch and Belgium zoos. We suggest a close monitoring of all avian populations within the zoos to establish whether the observed trend in this survey is still continuing and whether this is due to an actual increase in transmission.

Avian malaria: an emerging infectious disease in Europe?

In conclusion, could avian malaria potentially reach epidemic levels of avian malaria in Europe? Fact is that Hawaii is not a unique event; New-Zealand is currently struggling with avian malaria outbreaks after the introduction of *Cx. quinquefasciatus* three decades ago (Tompkins and Gleeson 2006). The same mosquito species also invaded the Galápagos Islands two decades ago, where the malaria parasite is, for the time being at least, still absent (Whiteman *et al.* 2005). However, with the increase of international travel, there is a high probability that parasites will be introduced to the Galápagos Islands at some point, which may potentially result in the same devastating effects as seen on the Hawaiian Islands. That would have devastating consequences for the biodiversity of these unique islands (Daszak *et al.* 2000).

An emerging disease only occurs when the hosts are highly susceptible. There are three scenarios in which a parasite is likely to have such an impact that a disease is gaining epidemic proportions. The first scenario is that a new and naïve host is introduced in a disease endemic area, as is the case with the black-footed penguins in our field study of Rotterdam zoo. Second, when a new parasite is introduced in a naïve bird population, such as on the Hawaiian Islands. Third, when due to adaptation or mutation, a parasite is suddenly capable of infecting a new host that it was unable to infect before or has gained an increased virulence. There are many examples of the latter in other diseases, such as the HIV/AIDS virus (Jaffar *et al.* 2004) and the Chikungunya virus in Reúnion (Schuffenecker *et al.* 2006).

It is difficult to predict whether future introductions of avian malaria in Europe should be considered as a threat of an emerging infectious disease, since there are few data available about the prevalence and especially the mortality rate of avian malaria in Europe. Therefore, the impact of the disease on the avian fauna on this continent is difficult to estimate. At the moment, a future outbreak of avian malaria is unlikely to happen; all endemic birds appear to be adapted to the parasites with whom they come into contact. However, a mutation in the existing parasite population can render it highly virulent to avian hosts. Also, a change of abiotic factors can affect the equilibrium between parasite and host. A change in climate for instance, may result in a longer transmission season or an increased transmission pressure, to which the birds might not be able to adapt to quickly enough. Also, new favourable climatic conditions might result in the spread of the parasites to a larger geographic area, where they will come into contact with new and possibly susceptible naïve hosts. Like in Hawaii, an introduction of a new, more competent vector can largely increase transmission of avian malaria. In other words, in a stable environment with regular host-parasite interactions it is unlikely that an avian malaria epidemic will occur. However, a change of the equilibrium between parasite and host or the emergence of new and virulent parasite strains or competent vectors could result in an epidemic among European endemic birds. It is therefore important to screen the avian population regularly, both migratory and endemic, so that intervention methods can be put into action before an outbreak reaches epidemic proportions.

The ecology and evolution of infectious diseases is an emerging research field. Emerging infectious diseases have drawn much attention lately, since they affect our health or that of our crops or livestock (Weiss and McMichael 2004). It is important to understand the principles behind the spread of emerging infectious diseases and to study host-parasite co-evolution. The study of avian malaria parasites will contribute much to our knowledge, and can be used as a model for other emerging infectious diseases. The introduction and spread of the avian malaria parasites in Hawaii and the subsequent evolution between parasite and host is a schoolbook example of what can happen if a virulent parasite enters a susceptible population. The recent past and the near future of the endemic Hawaiian bird population will teach us how the birds evolve to gain resistance and adapt to their new pathogens. Nevertheless, it resulted in an irreversible loss in biodiversity.

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