3. Wildlife research in the Netherlands

3.1. Avian Malaria in Rotterdam Zoo.

By: Willem Takken[1], Silvie Huijben[1], Adriaan Wijsman[1], Krijn Paaijmans[1], Harald Schmidt[2] and Willem Schaftenaar[2].

1 - Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands.
2 - Rotterdam Zoo, PO Box 532, 3000 AM Rotterdam, The Netherlands

Avian malaria is a parasitic disease caused by a variety of *Plasmodium* species (Figure). Historically, avian malaria has been responsible for the near extermination of indigenous birds on the island of Hawaii, USA, following the accidental introduction of *Culex quinquefasciatus* mosquitoes and avian *Plasmodium* spp. (mainly *P. relictum*). In more recent years, it has become clear that avian malaria is widespread, occurring globally both in temperate and tropical regions. Twenty-three species of avian malaria parasites have been described, distributed in 4 families (Garnham, 1966). Whereas mosquitoes of the genus *Anopheles* transmit human malaria species, avian malaria parasites are mostly transmitted by *Culex* spp., of which *C. pipiens* and *C. quinque-fasciatus* are the best known. Because both mosquito species are widespread, with sometimes high densities in urban areas, transmission of avian malaria parasites can occur frequently and with great intensity. Avian malaria has been reported from a large number of bird species, and does not seem to be restricted to a particular genus or family. The parasites may circulate in birds at a relatively high prevalence, but the impact of the parasites on the health of such birds is not well understood. Some species may suffer high losses as a result of infection, while others may be more tolerant.

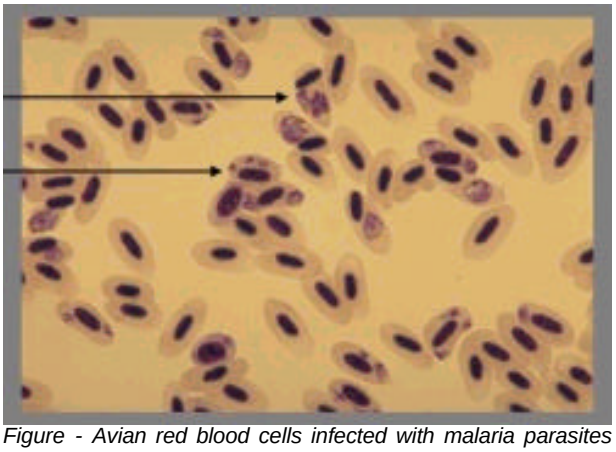

Figure - Avian red blood cells infected with malaria parasites (arrows) (from S. Jarvi and C. Atkinson, PIERC /PCSU project at UH Manoa. Hawaii)

Avian malaria in Rotterdam Zoo

Avian malaria has become associated with exotic birds kept in captivity. Various zoos around the world have reported illness and death of penguins affected by avian *Plasmodium*. In Rotterdam Zoo, avian malaria has affected black-footed penguins (*Spheniscus demersus*) and marine birds from the Northern Hemisphere including puffins. Several of these birds were housed in the recently opened Oceanium, an exposition of marine wildlife located in the north-western section of the zoo. The disease was reported to strike mostly in the summer months, and the incidence and virulence appeared to vary between years. It was thought that a high mosquito density, originating from ponds and lakes surrounding the Oceanium might be the reason for the unusually high incidence of avian malaria. To investigate the nature of avian malaria in Rotterdam Zoo, it was decided to initiate a pilot research project that would answer some of the questions relating to mosquito fauna and parasite transmission.

Research methodology

From May to September 2003, all sites containing water within and surrounding Rotterdam Zoo were inspected for the presence of mosquito larvae. Early in the study, 25 sites were selected for weekly inspection of mosquito larvae. Larvae were collected with a dipper, and the species and age composition of each sample determined. Adult mosquitoes were collected using odour-baited traps (MOSQUITO magnet, American Biophysics Corporation, Greenville, Rhode Island, USA). The odour bait consisted of a mixture of carbon dioxide and 1-octen-3-ol. Traps were emptied 2x per week, and adult mosquitoes were identified to species. Blood samples were taken from birds that were reported ill during the study. In addition, 120 blood samples were collected from apparently healthy birds, selected at random. Blood smears were examined microscopically for the presence of malaria parasites. In addition, each sample is currently being tested for *Plasmodium* parasites using a newly developed PCR technique (courtesy Dr. T.C. McCutchan, National Instutes of Health, USA and Dr. J.J. Verweij, LUMC, Leiden, The Netherlands). A sub-sample of adult mosquitoes collected during the study will also be investigated for the presence of malaria parasites.

Preliminary results of the pilot study

A large number of larval and adult mosquitoes was collected during the study period. The samples consisted mostly of culicine mosquitoes, with *Culex pipiens* and *Culiseta annulata* being dominant. Mosquitoes were found mostly within the zoo, but a

complex of vegetable gardens across from the Oceanium also produced numerous mosquitoes. A trap, placed inside the bird cage within the Oceanium, attracted considerable numbers of mosquitoes. By contrast, mosquito larvae were rare or absent in puddles and ponds near the Oceanium, indicating that mosquitoes arriving here must have immigrated from elsewhere. One small ornamental pond near the entrance to the Oceanium contained very large numbers of *Culex pipiens*, until the pump broke and the pond dried up. *Plasmodium* species were detected in at least nine penguins, while several other birds are suspected of carrying a *Plasmodium* infection. Of the 120 random samples of bird blood, malaria parasites were found in 13 samples. These birds did not show clinical signs of malaria at the time of blood collection.

Discussion

It is clear that avian malaria was present in Rotterdam Zoo in the summer of 2003. Unlike other years, this year only penguins appeared to be affected, with several deaths. As the blood samples are still being investigated for more accurate determination of malaria parasites and species identification, it is too early to conclude which avian *Plasmodium* species affected the birds, and what the prevalence of the disease was in 2003. Also, a PCR study on the mosquitoes can hopefully indicate which mosquito species was responsible for the infections. Current literature suggests that *Culex pipiens* should be considered as the vector, but it would be interesting to know whether other species can also be considered a vector.

The results of the study should assist in developing methods that will prevent further transmission of the disease in the zoo. Mosquito control will be prominent in this intervention, as several biological control options are available that should alleviate the problem. The completion of the study will also reveal the identity of the *Plasmodium* species circulating in the zoo. This leaves open the question of the origin of the parasites. Future studies should reveal whether the infections originate from migratory birds, or from resident, non-migratory birds that have become adapted to the parasitic infection.

References

Garnham, P.C.C. (1966) Malaria parasites and other Haemosporidia. Oxford, Blackwell Publishers. 1114pp.

3.2. Ecological effects of cyanobacterial toxins

by: Dr. K. Wolfstein
Email: k.wolfstein@riza.rws.minvenw.nl

Since 1996, the Institute for Inland Water Management and Waste Water Treatment (RIZA) has studied the ecological effects of cyanobacterial toxins in the lake called Ijsselmeer in The Netherlands. Monitoring of relevant parameters is carried out on the IJsselmeer, and experimental research has been done on the effects of cyanotoxins on zooplankton, zebra mussels (*Dreissena polymorpha*) and fish. The experimental research will be finalized in 2003.

Preliminary results show that toxic cyanobacteria are eaten by zooplankton and mussels. The measured microcystin levels in seston (i.e., organic and inorganic matter suspended in seawater), phytoplankton, zooplankton and mussels in the IJsselmeer are so high that intoxication of grazers and their predators can be expected. However, the interpretation of the values is based on the toxicity of a very toxic microcystin (microcystin-LR), and in the IJsselmeer other not yet identified microcystins occur, which, at least for water fleas (*Daphnia* sp.), are less toxic than microcystin-LR. Further research on the identity and toxicity of these microcystins is necessary.

To date, slow growth of zooplankton has been observed, as well as liver degeneration in fish in the IJsselmeer, but a clear cause-effect relationship to cyanobacterial toxins has not been established yet. Mussels seem to be little affected by cyanotoxins. They obviously possess mechanisms that reduce intoxication by cyanotoxins. However, it cannot be excluded that cyanotoxins are being transformed, and that they may therefore be missed in standard tests, which determine microcystin levels. There are also indications that microcystin levels in organs of animal origin are being underestimated because covalent-bonded microcystins could not detected. Therefore, the testing procedure is being improved at the moment.

RIZA is currently running the tests to determine whether the bird casualties this summer on the lake Volkerak-Zoommeer and the Oostvaardersplassen were caused by cyanobacterial toxins (microcystins or anatoxin). RIZA collaborates in this research with CIDC-Lelystad. The results are pending.