

## ABSENCE OF HAEMATOZOA IN BREEDING MACARONI *EUDYPTES CHRYSOLOPHUS* AND ROCKHOPPER *E. CHRYSOCOME* PENGUINS AT MARION ISLAND

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Haematozoan infections cause the death of penguins in captivity, but seldom in the wild. No haematozoa were found in 89 blood smears taken from macaroni penguins *Eudyptes chrysolophus* or 80 smears from eastern rockhopper penguins *E. chrysocome filholi* at subantarctic Marion Island between October and November 2001. Discussion centres on the possibility of vector introduction and establishment under conditions of climatic and/or anthropogenic change.

Key words: *Eudyptes*, haematozoa, Marion Island, penguins, Subantarctic

Blood parasitism is a subject of increasing interest (Hamilton and Zuk 1982, Earlé *et al.* 1991, Bennet *et al.* 1995, Jovani *et al.* 2002), resulting in extensive documentation of both host and parasite species throughout the world. Five species of haematozoa have been reported in penguins in the wild: *Leucocytozoon tawaki*, *Plasmodium relictum*, *P. elongatum*, *P. cathemerium* and *Babesia piercei* (Jones and Shellam 1999a). Haematozoan infections have been implicated as a cause of mortality in captive penguins and could therefore have an effect on the health of wild populations (Brossy 1992, Jones and Shellam 1999b). This study evaluates the occurrence of haematozoa in breeding macaroni *Eudyptes chrysolophus* and eastern rockhopper *E. chrysocome filholi* penguins at subantarctic Marion Island in the southern Indian Ocean. The Antarctic and Subantarctic have been inadequately studied in terms of blood parasites. The environment is thought to lack suitable vector populations, but various factors could act to increase the suitability of these habitats to the vectors of blood parasites.

### MATERIAL AND METHODS

Totals of 356 000 pairs of macaroni penguins and 67 000 pairs of rockhopper penguins (Crawford *et al.* 2003) breed on Marion Island (290 km<sup>2</sup>; 46°52'S, 37°51'E). Fieldwork was conducted at the Bullard Beach and Rockhopper Bay penguin colonies on the eastern side of the island between 3 October and 9

November 2001. Totals of 89 adult macaroni penguins (50 males, 39 females) and 80 adult rockhopper penguins (53 males, 27 females) were captured on return to their breeding colonies after the non-breeding winter period, and sampled for haematozoa.

Blood was obtained from the jugular vein and thinly smeared. Upon drying, Kyroquick (100% analytic-grade methanol) was applied for five seconds. Smears were transported to South Africa where, during August 2002, they were fixed with May Grunwald's Giemsa and stained with 4% Giemsa solution buffered to pH 7.3 (1/25 v/v) for 30 minutes, rinsed with 7.3 pH buffer and dried overnight. Blood smears were examined by light microscopy, for 15–20 minutes for each smear, with a minimum of 1 100 fields (c. 122 red cells per field) under 100× magnification oil-immersion objective for haematozoa.

During blood collection, ticks were opportunistically collected from the head of the penguins and preserved in 70% ethanol.

### RESULTS

No haematozoa were found in any of the 89 blood smears from macaroni penguins or the 80 smears from rockhopper penguins. Six ticks were found and identified as *Ixodes (Ceratixodes) uriae*. All were found on rockhopper penguins, four around the eyes and two around the base of the culmen. Tick burdens were low (6.7% of individual rockhopper penguins).

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## DISCUSSION

The lack of avian haematozoa in blood smears cannot be explained in terms of temporal or inappropriate sampling or methods used. Once infected, avian malaria infections remain with adult penguins throughout their lives, although they are not always detectable in blood smears (Cranfield *et al.* 1994). Chicks receive natural resistance of "anti-*Plasmodium* species" through maternal transfer for up to eight weeks after hatching (Graczyk *et al.* 1994). Therefore, only adult penguins were sampled. Justified concern has been expressed by Cooper and Anwar (2001) and Jones and Shellam (1999a) regarding procedures, methods and findings in reports interpreting parasitological examinations of blood smears as negative. Ideally, definitive assessment for malaria should be determined using a serum antibody ELISA test or polymerase chain reaction (Graczyk *et al.* 1995).

In captivity, avian malaria caused by the haematozoan *Plasmodium* spp. is the largest cause of mortality among penguins in American zoos (Cranfield *et al.* 1994). The same applies at the South African Foundation for the Conservation of Coastal Birds rehabilitation centre in Cape Town, in years when a major oil spill event affecting African penguins *Spheniscus demersus* does not occur (SLP pers. obs.).

There have been a few reports of haematozoa-induced mortality in the wild, but the intensity and the prevalence of these infections have been low (Jones and Shellam 1999a). *Leucocytozoon tawaki* (vector: simuliid flies) has been reported in Fiordland *E. pachyrhynchus* and African penguins; *Plasmodium* spp. (vector: mosquitoes and midges) in Fiordland, yellow-eyed *Megadyptes antipodes* and African penguins (Clarke and Kerry 1993, Jones and Shellam 1999a); and *Babesia peircei* (vector: ticks) in African penguins (Earlé *et al.* 1993). It is interesting that species where haematozoa have been identified in the wild are essentially "mainland" species breeding among vegetation where the environment is suitable for the establishment of vector species. There have been reports of negative blood examinations of 12 penguin species in their natural environment (700 birds from 14 localities, Jones and Shellam 1999a). No avian malaria was found in Galapagos penguins *S. mendiculus* (Miller *et al.* 2001).

Blood samples from penguins have been examined for haematozoa from several Subantarctic localities: Macquarie Island (Laird 1952, Jones 1988), Heard Island (Jones 1988) and South Georgia (Pierce and Prince 1980). All of these were negative. A northern rockhopper penguin *E. c. moseleyi* from cold-temperate Gough Island, South Atlantic, was reported positive

for *Plasmodium relictum* (Fantham and Porter 1944). However, no mention was made of where sampling took place, in the wild or in transit. Because no malaria vector species are present on Gough Island (Jones *et al.* in press), it is presumed that infection took place in transit.

The absence of blood parasites in penguins at Marion Island is attributed to the lack of established vector populations there. Similarly, the lack of haematozoa in birds living in the Arctic tundra has been attributed to the lack of appropriate vectors (Bennett *et al.* 1992, Earlé and Underhill 1993). Vectors commonly associated with genera of avian blood parasites include biting midges *Culicoides* spp., blood-sucking flies *Simulium* spp., mosquitoes *Culex* spp. and ticks *Ixodes* spp. All except the tick *I. uriae* are absent from Marion Island (Chown *et al.* 2002). Island habitats are, however, suitable for these genera in terms of physical environment and nutritional requirements. Dispersion to the island is possible through movement in the upper air column or through anthropocentric or avian introductions. Based on a few band recoveries (Gartshore *et al.* 1988), Subantarctic skuas *Catharacta antarctica* can reach the coast of Africa, where they are potentially exposed to avian malaria and *Babesia* through their vectors. These birds could then introduce infected vectors or infect vectors already established on Marion Island or on other subantarctic islands. No blood parasites were, however, found in a survey of south polar skuas *C. maccormicki* (Jones *et al.* 2002). Strong winds, cold temperatures and the absence of freshwater inhibit the establishment of vector species. Although there is an abundance of freshwater, Marion Island experiences winds exceeding 55 km h<sup>-1</sup> on c. 107 days per year. Atmospheric temperatures are usually between -2.8 and 13.9°C with an annual mean of 5.7°C (Hänel and Chown 1998). *Limnophyes pusillus*, an invasive chironomid midge, has established itself on Marion Island (Crafford 1986). Its success has been attributed to the suitability of its life history: short reproductive phase and loss of swarming behaviour (Crafford 1986). Future success of avian blood parasites and/or vector species introduced to Marion Island depends on their ability to adapt to prevailing environmental conditions. *L. pusillus* has been capable of overcoming such obstacles (Crafford 1986). Changing climate has impacted Marion Island. The mean annual temperature has increased by 1°C over the past 50 years and annual precipitation has decreased by 600 mm (Smith and Steenkamp 1990, Smith 2002). Climatic changes within the Antarctic and Subantarctic may increase the likelihood of establishment of alien insect species (Chown *et al.* 1998), including parasitic vectors.

Low tick infestation observed on rockhopper pen-

guins and the absence of ticks on macaroni penguins in this study may be attributed to the fact that these penguins were returning from six months at sea when sampled. In a previous study conducted at Marion Island, moderate tick infestations of rockhopper and macaroni penguins were observed (Brooke 1985). Moderate infestations of rockhopper penguins and massive infestations of royal penguins *E. schlegeli* were found on Macquarie Island (Murray and Vestjens 1967). Heavy infestations on king penguin *Aptenodytes patagonicus* chicks have been seen at Marion Island (J. Cooper, Avian Demography Unit, University of Cape Town, pers. comm.). Differences in severity of tick infestations between penguin species are determined by the duration of contact with plants and differences in breeding and moulting behaviours (Murray and Vestjens 1967). Acquired natural resistance to ticks is also a possible explanation for low infestation rates (Trager 1939, Roberts 1968), but this does not happen in all species (Randolph 1975). Serological studies could determine this relationship. *Ixodes uriae* is a vector for *Babesia peircei* (Earlé *et al.* 1993). *B. peircei* could therefore parasitize *Ixodes* spp. already established on Marion Island, potentially leading to deleterious consequences for penguin populations.

*Hepatozoon albatrossi*, also considered to be transmitted by *I. uriae*, has been recorded in wandering *Diomedea exulans*, grey-headed *Thalassarche chrysostoma* and black-browed *T. melanophris* albatrosses at South Georgia (Jones 1988). Wandering and grey-headed albatrosses both breed at Marion Island (Crawford *et al.* 2003). It is possible that this haematozoan is present in these species at Marion Island. *H. albatrossi* is considered non-pathogenic.

Subantarctic penguins are adapted to an environment free of haematozoan vectors (Laird 1961). Therefore, they have not developed an immune system to deal with these pathogens. If, as a result of global climate change or because of anthropogenic or avian introductions vectors become established on subantarctic islands, acute infections and mass mortality could occur, as has happened to birds on the oceanic island of Hawaii (van Riper *et al.* 1986). This underlines the need to prevent introduction of haematozoa and vectors to Marion Island.

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A view of the colony of macaroni penguins at Kildalkey Bay, Marion Island (photo B. M. Dyer)