# Treatment of and Pathological Findings of Leucocytozoonosis in a Fiordland Crested Penguin *(Eudyptes pachyrhynchus)*.

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**Abstract**

In November 2006, a Fiordland crested penguin of unknown sex and age, was found ashore on Muriwai Beach in the Auckland region of New Zealand. This is well outside of its normal range. On examination the bird had pale mucous membranes, a cloacal temperature of 40.5ºC, was underweight, and appeared weak. Examination of a blood sample revealed a severe anaemia. A blood smear demonstrated large numbers of *Leukocytozoon spp.* parasites and occasional intracellular structures in the red blood cells (RBC), that were presumed to be either a *Babesia spp*. or *Plasmodium spp*. A diagnosis of *Leukocytozoon spp.* infection was made based on the presence of large numbers of organisms in the blood smear. The RBC parasites were considered to be of less significance. Treatment with a trimethroprim/sulpha combinations was instituted and continued for a period of three weeks. During this time the penguin made a full clinical recovery. *Leukocytozoon spp.* parasites were not seen in a blood smear after the end of treatment. Despite the apparent success of the treatment, the penguin subsequently died. This case report discusses the various post mortem findings and their significance.

**Figure 1 - Fiordland Crested Penguins**

**Case report**

In November 2006, a Fiordland crested penguin of unknown sex and age was found ashore on Muriwai Beach in the Auckland region of New Zealand. This is well outside of it's normal range. It was brought to a volunteer-run, bird rehabilitation centre in Auckland. Initially the bird was given supportive care with gavage feeding but remained depressed and failed to gain weight. Veterinary intervention was sought one week after capture.

The bird was in poor body condition (weight not available) and had pale mucous membranes. It was weak and depressed. It's cloacal temperature was 40.5ºC. A blood sample was collected and submitted to a commercial veterinary laboratory.

Serum chemistry and complete blood count (CBC) results are shown in Table 1.

**Table 1. Haematological and serum biochemical values**

| | 8th Nov2006 | 18th Nov 2006 | 15th Dec 2006 | Reference range | Units |
|---|---|---|---|---|---|
| WBC | 9.1 | 14.7 | 6.3 | 1.6 - 25.1 | $x10^9$/L |
| Lymphocytes % | 25 | 24 | 36 | | |
| Lymphocytes abs. | 2.3 | 3.5 | 2.3 | 0.61 - 14 | $x10^9$/L |
| Monocytes % | 0 | 3 | 2 | | |
| Monocytes abs | 0 | 0.4 | 0.1 | 0.016 - 2 | $x10^9$/L |
| Eosinophils % | 0 | 2 | 3 | | |
| Eosinophils abs | 0 | 0.3 | 0.2 | 0.05 - 2.65 | $x10^9$/L |
| Basophils % | 1 | 2 | 4 | | |
| Basophils abs | 0.1 | 0.3 | 0.3 | 0.03 - 9.59 | $x10^9$/L |
| Heterophils % | 74 | 69 | 55 | | |
| Heterophils abs | 6.7 | 10.1 | 3.5 | 0.61-18.8 | $x10^9$/L |
| HCT | 17 | | 31 | 35 - 59 | % |
| CPK | 1168 | | | 150 - 2311 | IU/L |
| AST | 695 | | | 59 - 828 | IU/L |
| ALT | 95 | | | 10 - 245 | IU/L |
| ALP | 90 | | | 0 - 237 | IU/L |
| TP | 38 | | | 29 - 64 | g/L |
| Phosphorus | 1.4 | | | 0.1 - 3.4 | mmol/L |
| Ca | 2.43 | | | 1.8 - 3.1 | mmol/L |
| Glucose | 11.7 | | | 9.4 - 20 | mmol/L |
| Uric acid | 1113 | | | 101 - 1886 | mcmol/L |

Reference ranges taken from published data on the rockhopper Penguin (*Eudyptes crestatus*) - International Species Information System, March 2002

Serum chemistry results were all within normal range when compared with reference ranges for the rockhopper penguin (*Eudyptes crestatus*).

The CBC revealed a severe anaemia (HCT = 17) and normal white cell count (WBC = 9.1). The differential white cell analysis revealed a predominantly heterophilic population (74%). A blood smear examination demonstrated large numbers of leukocytozoon parasites within blood cells. There were also small numbers of intracellular red blood cell structures. These were thought to be *Babesia spp.* or *Plasmodium spp.* but the numbers were not considered significant.

Based on the anaemia and the large numbers of parasites seen, a diagnosis of leukocytozoonosis was made. Treatment with trimethoprim/sulpamethoxazole (Trisul Paediatric Suspension, Pacific Pharmaceuticals) was initiated at a dose rate of 30mg/kg twice daily. Later, due to practical issues with dosing, the medication was changed to trimethoprim/sulfadiazine (Tribactril 80 tablets, Jurox) at the same dose rate.

After ten days of treatment, the penguin appeared brighter with an improved appetite. A repeat blood sample was collected and submitted to the same laboratory (Table 1). The PCV was not measured, however a blood smear was evaluated. At this time there were still large numbers of leukocytozoon parasites however the pathologist felt that the parasites appeared to be degenerating. Treatment was continued unchanged for a total of 3 weeks.

A further 4 weeks later, a third blood sample was analysed (Table 1). At this time the PCV was 31 and the laboratory could find no evidence of leukocytozoon in the blood smear. PCR of peripheral blood was negative for leukocytozoon. Clinically the penguin was bright, with a very good appetite and it's body condition was judged to be normal. The treatment was deemed to have been successful and supportive care continued at the rehabilitation centre until a release date could be arranged.

Approximately 4 weeks after the last blood sample, the penguin developed a sudden onset depression (as described by the care-givers) and died before veterinary attention could be obtained. A post mortem examination was carried out however no diagnosis was reached on gross examination. Histopathology revealed a multifocal cholangiohepatitis characterised by heterophils and macrophages; severe, localised interstitial pneumonia and pulmonary collapse; and localised renal coccidiosis with moderate to severe tubular degeneration. Leucocytozoon schizonts or megaloschizonts were not seen. Occasional leucocytozoon gametocytes were noted within visceral vessels. It was not clear whether any, or all of these conditions contributed to the death.

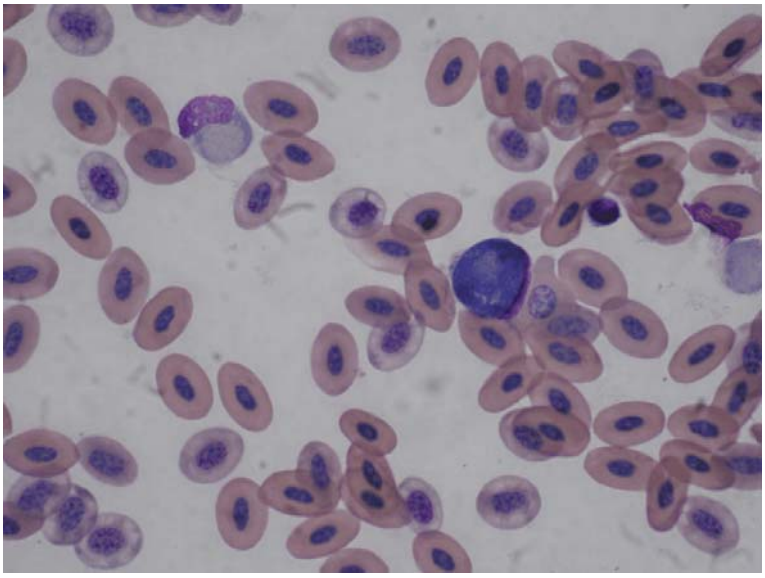

**Figure 2. Blood smear showing a leukocytozoon gametocyte**.

**Discussion**

Fiordland crested penguins are a vulnerable, endemic species to New Zealand, nesting on the South-west coast of the South Island and Stewart Island. Vagrants have been found as far afield as Western Australia, 4000km away. Population estimates range up to 3000 pairs but little is known about population trends. They are the endemic hosts of *L.tawaki* within their natural range (Fallis *et al* 1976).

Leucocytozoon is an obligate intracellular protozoal haemoparasite that replicates within avian endothelial cells and hepatocytes. Gametocytes are found in red blood cells. Species tend to be host specific (Bennet *et al* 1992), and have been reported in Fiordland and Yellow-eyed penguins in New Zealand (Fallis *et al* 1976; Alley 2005). Leucocytozoon has also been documented in captive African and Macaroni penguins (Peirce *et al* 2005), and had significant impacts on domestic poultry (*L.caulleryi*, *L.smithi*) and waterfowl (*L.simondi*) worldwide (Atkinson *et al* 1991, Fallis and Desser 1977, Greiner and Ritchie 1994, Herman *et al* 1975)

Infections range from subclinical to fatal. Sdigns of leukocytozoon infections include weakness, anaemia and sudden death (Atkinson *et al* 1991). Other signs include tachypnoea, petechial haemorrhage, diarrhoea, biliverdinurea, and neurological signs. Flock infections can manifest as seasonal or sporadic epizootic mortalities (Atkinson *et al* 1991). Juveniles and immunosuppressed adults are most susceptible. Clinical leucocytozoonosis of pathogenic species is thought to be caused by a combination of antierythrocytic factors, gametocytes blocking capillaries, and invasive tissue phases causing multifocal necrosis (Cowan 1957, Kocan 1968, Greiner and Ritchie 1994, Khan and Fallis 1970, Maley and Desser 1977).

Infectivity has a strong environmental component including vector density, immunosuppression and prior infection (Allison *et al* 1978; Jones and Shellam 1999). Transmission studies identified the simuliid fly *Austrosimulium ungulatum* as the sole vector

in Fiordland crested penguins within their natural range (Allison *et al* 1978). Their peak feeding period was September to November during the breeding period, coinciding with peak infection rates. Infections were not fatal, but may have predisposed infected birds to secondary disease. Heavy parasitaemias were seen in chicks and lighter burdens in adults (Allison *et al* 1978).

Inoculated sporozoites form schizonts within hepatocytes and macrophages, undergoing asexual reproduction until the host cell ruptures (Desser and Fallis 1967). Merozoites are released and infect erythrocytes and leucocytes, or are phagocytosed by macrophages and endothelial cells to produce large protozoal cysts (megaloschizonts) up to 80um diameter, in the liver, spleen, brain and kidney. Re-activation of latent infections is thought to occur with immunosuppression due to stress, concurrent infections or glucocorticoid administration (Atkinson *et al* 1991). In fact, the level of parasitaemia may indicate the severity of underlying diseases (Remple 2004). It is proposed that due to the marked tissue phase of leucocytozoon compared to plasmodium, recrudescence after immunosuppression or treatment is more likely (Gill and Paperna 2005).

Clinical pathology of leucocytozoon is non-specific. Anemia is common and leucopaenia may be present in some cases (Maley and Desser 1977). Blood smears provide a simple and rapid diagnosis (Desser *et al* 1968), however, Gill and Paperna (2005) found that examination of peripheral smears alone may not be sufficient to determine the presence of some leucocytozoon infections. Further diagnostics include PCR and histopathology. Identification of tissue phases in the liver, spleen and kidney provide definitive diagnosis but are difficult to acquire antemortem. Diagnosis of wild birds is usually at post mortem during flock mortalities.

Multiple treatment strategies have been suggested, but few have proved reliably successful. Primaquine and chloroquine have been used to address combined *Plasmodium* /*Leucocytozoon* infections in a range of species but is not highly successful in clearing leucocytozoon (Evans and Otter 1998). Other strategies include melarsomine (Tarello 2006), quinacrine HCl (Cooper 1985) and trimethoprim/sulfamethoxazole (Remple 2004) in raptors. The use of trimethoprim and sulfamethoxazole in a peregrine falcon dramatically reduced, but did not eliminate, parasitaemia (Remple 2004). We found that a similar treatment protocol apparently resulted in an increased PCV and suppressed parasitaemia to levels not detectable on blood smears. Histological evidence of gametocytes in blood vessels, however, indicated a failure to completely eliminate infection. It remains unclear what effect this treatment may have on the tissue phase of disease.

This individual was infected with multiple haematozoa and infection with a secondary organism may have contributed to its clinical signs. Babesiosis is uncommon and natural infections have been documented in penguins, but little is known of their significance (Brossy *et al* 1999). Plasmodium is widely documented as a cause of death in captive penguins, but endemic infections in wild penguins does not necessarily cause disease (Duignan 2001).

Despite what appeared to be a successful treatment, this bird died during rehabilitation. Post mortem and histological findings did not provide a definitive cause of death. Cholangiohepatitis and interstitial pneumonia most likely represent secondary diseases in

captivity. Pulmonary disease in hospitalised seabirds is very common, however this bird did not show clinical signs and the pneumonia was localised. Renal coccidiosis has been identified in little blue penguins and is often an incidental finding (Rose K, 2005). No single lesion appeared sufficient to cause death.

This report describes a case of a penguin infected with multiple infectious agents. It highlights the role of endemic infections in clinical disease and some of the obstacles facing the practitioner treating wildlife. The combination of antibiotic therapy and supportive care may have had multiple beneficial effects including suppressing concurrent bacterial infection. It is likely that suppression of the parasitaemia during treatment was a factor in the initial improvement in the penguin's condition. It is unclear whether these drugs are able to cure a patient of leukocytozoonosis. However, with wide availability and good margin of safety, their use can be advocated as an aid in recovery from this infection.

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