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Melioidosis in a macaroni penguin *Eudyptes chrysolophus**

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ABSTRACT: A female macaroni penguin *Eudyptes chrysolophus*, at Ocean Park, Hong Kong, died suddenly during a period of extremely high ambient temperature and humidity. Necropsy results including pathological, histological, and microbiological observations indicated that the penguin died due to disseminated *Pseudomonas pseudomallei* infection. This is the third report of this disease in avian species, and the first report involving a penguin.

INTRODUCTION

Melioidosis has been recognized since Whitmore's classic account of the disease in Rangoon (Whitmore 1913), and is still important in endemic areas (Chaowagul et al. 1989, Dance et al. 1989). There is a distinct geographic distribution of this disease, with most cases occurring between latitudes 20° N and 20° S. Melioidosis is of greatest importance in Australia and Southeast Asia, including Hong Kong. The disease has been reported from various continents and in numerous animal species, including humans (Vedros 1988). The etiologic agent *Pseudomonas pseudomallei* is present in the environment and may be cultured from water and soil in endemic areas (Strauss et al. 1969, Pourthaghva et al. 1975, Thomas et al. 1979).

CASE REPORT

This report concerns a case of melioidosis in a female macaroni penguin *Eudyptes chrysolophus* at Ocean Park, Hong Kong. *Pseudomonas pseudomallei* has been isolated from various animals at Ocean Park, including cetaceans (Liong et al. 1985). The penguin involved in this report was acquired from Chile 3.5 mo prior to its death. The animal was housed in an outdoor holding area with other penguins until it exhibited signs of environmental stress. At the first signs of heat exhaustion, the penguin was removed from the outdoor pen. The ambient temperature was approximately 29.4 to 32.2 °C, with a relative humidity of 90 to 95 %. The penguin was placed in an air-conditioned isolation room (approximately 22 °C) in the veterinary hospital. Clinical signs included anorexia, lethargy, slightly increased respiratory rate, and copious, light brown diarrhea. A slow recovery was observed over the following 10 d, and the penguin was returned to the outdoor penguin holding area. At this time, the penguin was demonstrating good feeding and activity patterns. Approximately 1 mo later, the penguin was found dead in the holding pen. There were no premonitory signs and the animal was considered to be in good body condition at the time of death. Because no disease was suspected, the penguin had not been under treatment.

A necropsy was performed within approximately 10 h of death and samples were collected for microbiological and histological examination.

Microbiology

At necropsy, swabs were aseptically collected from the right and left abdominal air sacs (fluid and scrapings), right and left kidneys, small and large intestines, proventriculus, spleen, right and left lungs, and right and left liver lobes. The swabs were placed in Stuart's transport media until they were plated onto culture media, immediately following conclusion of the nec-

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ropsy. Heart blood was collected and inoculated into aerobic and anaerobic blood-culture bottles. Swabs were plated onto the following agar media: heart infusion with 5% goat blood, eosin methylene blue, MacConkey, *Pseudomonas pseudomallei* selective medium, xylose lysine desoxycholate, thiosulfate citrate bile salts sucrose, V-9 fungal medium, and Hektoen enteric medium. Blood cultures were subcultured onto goat blood agar plates after 48 h incubation. All plated media were incubated 48 h at 35°C. Isolated colonies were cloned to pure culture for identification.

Isolate identification was performed using standard biochemical methods, and the API (Analytical Products, Inc.) system. Table 1 lists all of the organisms isolated from various sites.

Histology

Gross pathology. The overall macroscopic pathology suggested an acute septicemic condition. Pin-point creamy white lesions were observed on the surfaces and dispersed within parenchymal tissues of liver and lungs. Scattered petechiae were observed on the mucosal lining of the bronchi, trachea, pericardium, and caudal abdominal air sacs. Approximately 5 ml of a straw coloured fluid were collected from the air sacs. There were foci of dark brown areas of congestion in the proventriculus, and the small and large intestines.

Tissue samples collected from each organ at the time of necropsy were placed in 10% formalin saline until they were sectioned and stained with haematoxylin and eosin. Stained sections were observed for microscopic pathology and the presence of bacteria.

Microscopic pathology. Histologically, focal areas of inflammation were present in the lungs, liver, kidneys, caudal abdominal air sacs, and spleen. Small, circular, non-encapsulated areas of necrosis were found throughout the lung and liver tissue. Vascular engorgement, mild local oedema, and infiltration of neutrophils were the major characteristics of these inflammatory sites. Bacilli were observed in some sections.

DISCUSSION

Due to the isolation of various organisms from the different internal organs, it was not possible to state with certainty that *Pseudomonas pseudomallei* was solely responsible for the penguin's death. Also, the penguin was not necropsied immediately following death and some organisms may have migrated from their normal sites to the animal's viscera prior to the time of necropsy. The isolation of *P. pseudomallei* in pure culture from the heart blood, spleen, and liver, in addition to the isolation of this organism from every other site sampled does indicate the presence of disseminated disease. The evidence obtained from the

Table 1. *Eudyptes chrysolophus*. Organisms isolated from various sites

Site	Organism
Lung (right and left)	*Pseudomonas pseudomallei* *Aeromonas hydrophila* *Acinetobacter calcoaceticus* var. *lwoffi*
Kidney	*P. pseudomallei* *A. hydrophila* *Edwardsiella tarda*
Intestines (large and small)	*P. pseudomallei* *E. tarda* *Escherichia coli*
Proventriculus	*P. pseudomallei* *E. tarda* *Es. coli*
Abdominal air sacs (right and left, fluid and scrapings)	*P. pseudomallei* *Proteus vulgaris* *A. hydrophila* *Flavobacterium sp.* *Pseudomonas alcaligenes* (probable species) *Vibrio alginolyticus* (probable species)
Liver (right and left lobes)	*P. pseudomallei*
Spleen	*P. pseudomallei*
Heart blood	*P. pseudomallei*

pathological, histological, and microbiological examinations indicated that the penguin died due to disseminated *P. pseudomallei*. The observations in this case are consistent with melioidosis in humans, dolphins, and other species (Brundage et al. 1968, Greenawald et al. 1969, Jayanetra et al. 1975, Narita et al. 1982, Liong et al. 1985, Fritz et al. 1986, Thomas et al. 1988). Contributing factors in this penguin's disease included environmental stress (high temperature and humidity), and possible concurrent infection with other organisms. Other organisms isolated from this animal included *Edwardsiella tarda, Escherichia coli* and *Aeromonas hydrophila*, strains of which are avian pathogens (Arnall & Keymer 1975, Gerlach 1986).

An intriguing sidelight to this case is the fact that this was the only penguin at Ocean Park which died with *Pseudomonas pseudomallei*. Two other penguins of different species died due to aspergillosis at about the same time. These observations point to the opportunistic nature of *P. pseudomallei*.

In conclusion, *Pseudomonas pseudomallei* is ever present in the environment of endemic areas and is capable of causing very severe, rapidly fulminating disease in compromised animals. While other significant contributing factors were involved, *P. pseudomallei* was most the most likely cause of this penguin's death. This case is unique in that it is only the third report of melioidosis in an avian species (Thomas et al. 1978, Thomas et al. 1980), and the first report of this disease in a penguin.

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