

***Pseudomonas pseudomallei* infection in a dolphin (*Tursiops gilli*): A case study**

Edgard Liong, D. D. Hammond and Neylan A. Vedros*

*Ocean Park, Hong Kong and *School of Public Health, University of California, Berkeley, California 94720*

Summary

The pathology and microbiology, of a dolphin (*Tursiops gilli*) dying from melioidosis is presented as a case study. Measures to control and prevent the disease are briefly discussed.

Introduction

Melioidosis is a disease caused by the water and soil-borne microorganism *Pseudomonas pseudomallei* (Whitmore's bacillus). The disease is endemic to Southeast Asia (Strauss, Alexander, Rapmund, Gan & Dorsey 1969) but is now recognized in man and other animals worldwide (Dodin 1977). At the Ocean Park facility in Hong Kong, *Ps. pseudomallei* can be isolated from the soil, vegetation, water, and probably the air. Melioidosis has been one of the major causes of mortality in both cetaceans and pinnipeds over the last 5 years with the highest incidence during May–October. During this period the animals are under stress from high ambient temperatures and *Ps. pseudomallei* are abundant in the soil and water from the winter rain runoff.

The present report describes a typical case of melioidosis in a cetacean (*Tursiops gilli*) and a brief discussion of current efforts to control the disease.

Case Report

History. A female *T. gilli* (named KiKi) was captured on 25 February 1981 off IKI Island, Japan. The animal was given the standard Erysipelas bacterin while still in the sea pen and transported to Ocean Park on 17 March 1981. On arrival the animal was given Ampiclox (2 gm I.M.) q.d. followed by 2–500 mgm capsules t.i.d. for 7 days. The animal was bled periodically during 1981 and the range of haematological values are shown in Table 1. The range of values were similar to other *T. gilli* at Ocean Park and the animal was essentially normal in its eating and activity patterns.

On 4 August 1982, the animal was active but consumed only 13 lbs of her normal 19 lb of fish food.

She was not interested in the last feeding of the afternoon. On 5 August 1982, the animal was active but was not interested in food. The tank was drained and a blood sample drawn for analysis. The rectal temperature was 101°F. Because this was the time of the year when melioidosis occurs the animal was given Terramycin Q-100 (15 ml, I.M., q.d.); Dexamethasone (16 mgm, I.M., q.d.), Irgapyrin (3 ml, I.M., q.d.). As shown in Table 1 the haematological values were in the normal range for this animal except for a slight fall in the WBC. On 6 August 1983, at 8 am the animal was swimming with other dolphins in the pool but showing some respiratory distress. She became morbid at 8:20 am and died at 8:30 am. The necropsy was performed within 1 hr.

Table 1. Selected haematological values and *T. gilli* (KiKi)

Test	Dates of Bleedings*	
	25 Mar–7 Oct 81	5 Aug 82
Hb (gm/dl)	19.1–21.6	19.6
RBC (10 ⁶ /mm ³)	4.08–4.96	4.46
PCV (%)	0.50–0.57	0.56
WBC (M ³)	6,900–11,600	4,800
ESR	<1–5	5
Neut (Total %)	34–78	96
Eosin (%)	9–48	0
Baso (%)	0	0
Lymph (%)	10–21	3
Mono (%)	3–8	1
Total Protein	63–76	73
Alb (g/dl)	38–45	36
Glob (g/dl)	24–34	37
Bilirubin (mg/dl)	0.2–0.5	0.4
SGOT (U/l)	50–94	74
SGPT (U/l)	7–40	20
BUN (mg/dl)	11.7–22.6	9.1
Amylase	64–91	72

*The range of values for blood collected during 25 Mar–7 Oct 81 were derived from 8 bleedings at approximately monthly intervals.



Figure 1. Histologic section of left lung. H & E stain. 520 × magnification.

Necropsy

The animal measured 9 ft 7 in in length and 4 ft 6 in in girth. The age was estimated to be 8–10 years and the last body weight (2 August 1982) was 490 lbs. The pancreas, right lung, kidneys, and ovaries were normal. The heart was normal except for small petechial haemorrhages. The spleen was swollen with petechial haemorrhages and the stomach had a superficial ulcer. The left lung had a 25 cm active haemorrhagic abscess and a 5 cm chronic abscess. The liver had small abscesses plus petechial haemorrhages. The mesenteric lymph nodes were normal but the thoracic lymph nodes were swollen with no pus. The brain was not examined.

Histology

Tissue specimens were obtained from left lung, spleen, and liver for histologic preparation. A section of the lung is shown in Figure 1. As can be noted, a small microabscess is present in the vicinity of the tertiary bronchioles. The principle reactive cells were polymorphonuclear leucocytes, lymphocytes, and occasional monocytes. There was a tendency of the surrounding alveolar walls to contain large numbers of small, rounded cells. The thickening of the alveolar wall was due to infiltration. Although not visible at the magnification in Figure 1 selected congestion of small blood vessels was noted in the area of the microabscess and bacteria

could easily be seen concentrated in the microabscess.

Microbiology

Cultures were taken aseptically from all organs and plated on blood agar (aerobic and anaerobic incubation), *Ps. pseudomallei* selective medium, XLD medium, and EMB medium. Pure cultures of only *Ps. pseudomallei* were isolated from heart blood, both spleen and lung abscesses, kidney, and pancreas. Isolates from the stomach included *Ps. pseudomallei*, *Micrococcus sp.* and *Enterobacter sp.* Culture of the thoracic lymph nodes yielded *Ps. pseudomallei* and *Bacillus sp.* (not *B. anthracis*). Other enteric bacteria (*Escherichia coli* and *Enterobacter sp.*) were isolated from the large and small intestines but not *Ps. pseudomallei*.

The *Ps. pseudomallei* were sensitive in descending order to Vibromycin, Tetracycline, Chloramphenicol, Carbenicillin, Novobiocin, and Kanamycin.

Discussion

Melioidosis in man is an acute pulmonary infection with occasional, rapid progressive fatal septicaemia in spite of therapy. The dolphin described in this report is typical of the clinical manifestations seen in other cetaceans dying of melioidosis at Ocean Park. The onset is insidious with developing respiratory distress and death. The temperature is always elevated and the animal refuses to eat. The haematological values (Table 1) were not significant and this was noted in a number of cases. Generally the WBC count drops as seen in this case but the most consistent value noted was the albumin/globulin ratio. In our healthy cetaceans this ratio ranged from 1.5-2.5 but in approximately 50% of the animals dying from melioidosis the ratio dropped to 1.0 or slightly below. The significance of this observation is not known at present and requires further study: The gross pathology described in this report is similar to that seen in other animals except for differences in severity. In some animals the lungs were

heavily infiltrated with multiple abscesses possibly indicating the primary route of infection. Abscesses are also frequently seen in large numbers in the spleen and both the thoracic and mesentery nodes can be very swollen, necrotic, and full of pus. In about 30% of the animals haemorrhage was noted in the stomach lining.

The prevention and control of melioidosis in marine mammals at Ocean Park has been approached in several ways: (1) More frequent flushing and sterilization of the filters have been instituted and this has reduced the bacterial count in the effluent water significantly, (2) the level of chlorine is maintained at 1 PPM in the pools but it was noted that the bactericidal activity of the water against *Ps. pseudomallei* decreases with a decrease in salinity. This observation is currently under study, (3) a more effective antibiotic, cefoperazone, is being used and serum levels being determined, and (4) two experimental vaccines are being tested with promising results with a protein-polysaccharide antigenic fraction. The above approaches have resulted in the mortality due to *Ps. pseudomallei* being reduced to less than 1%. The disease however will always be a threat to marine mammals and other animals in the Hong Kong area, thus requiring continuous surveillance.

Acknowledgements

We gratefully acknowledge the help of the Ocean Park hospital staff and Professor S. H. Madin of the University of California, Berkeley, for examining the histological preparations.

References

- Dodin A. (1977) Aperçus nouveaux sur l'écologie du bacille de Whitmore. *Bull. Acad. Vet. de France*, **50**, 513-517.
- Strauss J. M., Alexander A. D., Rapmund G., Gan E. & Dorsey A. E. (1969) Melioidosis in Malaysia. III. Antibodies to *Pseudomonas pseudomallei* in the human population. *Amer. J. Trop. Med.* **18**, 703-707.