Cutaneous and Respiratory Tract Infection with Mycobacterium ulcerans in Two Koalas (Phascolarctos cinereus)

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TABLE 1. Prevalence of viral erythrocytic necrosis and *Ceratomyxa shasta* in pink salmon collected at six sites in the Fraser River system in 1983.

Site and date collected	Water temperature (C)	Viral erythrocytic necrosis[a] Prevalence (%)	Viral erythrocytic necrosis[a] Number of fish infected/number of fish examined	*Ceratomyxa shasta* Prevalence (%)	*Ceratomyxa shasta* Number of fish infected/number of fish examined
Fort Langley 22 Sept.	13.9	0	(0/30)	0	(0/30)
Yale 27 Sept.	13.0	13	(4/30)	0	(0/30)
Lytton 30 Sept.	12.8	40	(12/30)	0	(0/30)
Lillooet 4 Oct.	10.8	50	(15/30)	0	(0/30)
Ashcroft 5 Oct.	12.8	30	(9/30)	0	(0/30)
Seton spawning channel (Seton Creek) 14 Oct. (post-spawned)	10.8	23	(7/30)	20	(6/30)
Seton stamina tunnel[b] 14 Oct.	10.8	45	(17/38)		Not examined

[a] Presumptive diagnosis.
[b] An additional group from the run, used for swimming stamina tests.

cit.; Rohovec and Amandi, 1981, Fish Pathol. 15: 135–141; Sanders et al., 1970, Am. Fish. Soc. Spec. Publ. No. 5, pp. 133–141).

Voucher specimens of *C. shasta*, and erythrocytes containing putative ENV inclusion bodies have been deposited in the National Museum of Natural Sciences of Canada as NMPCPC1984-0808 and NMCPC1984-0807, respectively.

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Cutaneous and Respiratory Tract Infection with *Mycobacterium ulcerans* in Two Koalas (*Phascolarctos cinereus*)

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Cutaneous ulcers in man caused by *Mycobacterium ulcerans* occur in several specific localities around the world, including southeastern and northern Australia. These localities are usually centered on a river or estuarine system, and the causative organism is thought to reside in soil and water in these areas (Radford, 1975, Aust. N.Z. J. Med. 5: 162–169). The disease in humans is characterized by progressive ulceration and necrosis of the skin. Infection may occur in underlying muscle and bone, but has not been reported in the respiratory tract or other internal organs (Radford, 1975, op. cit.). In the first report of an animal other than man being infected naturally with *M. ulcerans*, Mitchell et al. (1984, Pathology 16: 256–260) described necrotizing ulceration of

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the skin in seven koalas on an island (Raymond Island) within an inland lake system in southeastern Australia. This island is within a locality where cutaneous ulcers due to *M. ulcerans* are known to occur in man (Radford, 1975, op. cit.).

Two further cases of koalas with deep ulcers in the skin were detected on Raymond Island. They were assigned to age class 5, by the methods of Martin (1981, Aust. Wildl. Res. 8: 275–283). The two koalas, one male, one female, were both in poor nutritional condition, with deep cutaneous ulcers, 5 to 20 mm diameter, on their faces. One koala had two similar ulcers on one front foot. The ulcers were deep craters in the skin, with a grey moist base. On midline sagittal sectioning of the skulls, the ulcers on the maxillae were seen to extend into the nasal turbinates, forming grey-yellow raised, roughened areas on the mucosa. The lungs had several pale grey soft masses 10–20 mm diameter, adjacent to large bronchi in the hilar region.

Histologic examination of the cutaneous ulcers showed coagulative necrosis of the dermal and epidermal structures. This necrosis extended into the dermis and subcutis beneath and around the ulcers' margins. At the edge of the ulcer, there was mild to moderate infiltration by macrophages and neutrophils, with some edema, hemorrhage and vascular thrombi. On Ziehl-Neelson staining, clusters of 1.2 to 1.5 μm long, slender, acid-fast bacilli were detected in necrotic areas, and within macrophages. Examination of the nasal turbinates showed mucosal infiltration by macrophages, neutrophils and lymphocytes, with some epithelial necrosis. Clusters of acid-fast bacilli of similar morphology were detected within macrophages. Examination of the pulmonary masses showed peribronchial infiltration with macrophages, lymphocytes and some epithelioid cells. Occasional acid-fast bacilli of similar morphology were detected in macrophages. Several associated foci of necrosis with surrounding neutrophils and macrophages were detected in lung tissue. In both the lung and nasal turbinates, the submucosal lymphoid follicles appeared hyperplastic, but were not infiltrated by other cells or bacilli. Examination of the livers showed numerous small granulomas consisting of macrophages and lymphocytes in the portal triads. No acid-fast bacilli were detected in these granulomas.

Numerous clumps of five to ten slender, acid-fast bacilli (1.2 to 1.5 μm long) were seen in fresh smears of the cutaneous ulcers and nasal turbinates. Occasional bacilli of similar morphology were seen in smears of affected portions of both koalas' lungs.

Small blocks of fresh tissue, incorporating the edge of the skin ulcers, the hilar region of the lungs and the nasal turbinates, were cut into small fragments and agitated with 3-mm glass beads in a vortex mixer. Fresh samples of the preparation were cultured onto Lowenstein-Jensen medium and incubated at 30 C and 37 C.

Mycobacterium ulcerans was isolated from cutaneous ulcer, nasal cavity and lungs of one koala. No other mycobacteria were isolated from either koala. The isolates were identified as *M. ulcerans* by their pale cream, rough, dry colonial appearance, absence of growth at 37 C and presence of growth at 30 C after 8 wk incubation, in air (Marks, 1976, Tubercle 57: 207–225).

Mycobacterium ulcerans has a preferred temperature for optimal growth of 30 C to 33 C (Marks, 1976, op. cit.); therefore, infection tends to occur in the cooler sites of the body, particularly the skin. Progression of cutaneous ulcers into underlying muscle and bone, has occurred in some human cases (MacCallum et al., 1948, J. Pathol. Bacteriol. 60: 93–122). However, the progression from the facial cutaneous ulcers into the nasal turbinates and lungs in these two cases is the first such occurrence reported in koalas. The hilar region of the lungs may have been kept to a suitable temperature for the

growth of *M. ulcerans*, by airflow over the region. The establishment of the *M. ulcerans* infections in the deeper tissues of these koalas may have been facilitated by the presence of a subnormal body temperature. The normal body temperature of a koala (37 C) is usually lowered to 29–30 C if the animal becomes sick or debilitated (Dickens, 1975, Aust. Vet. J. 51: 459–463). The presence of an indolent ulcer in the skin may have been sufficient to lower the body temperature of the affected koalas thus aiding infection of deeper tissues by *M. ulcerans*. The hepatic granulomas seen may have been a response to ingested mycobacterial antigens. However, they were not considered specific.

Cutaneous infection by *M. ulcerans* has been associated previously with coagulative necrosis and a mild neutrophil, macrophage response in both koalas (Mitchell et al., 1984, op. cit.) and man (Dodge, 1964, J. Pathol. Bacteriol. 88: 167–174). Marsupials infected with other mycobacteria have shown different responses. Experimental *M. bovis* infection of opossums produced a mononuclear cell response (Corner and Presidente, 1980, Vet. Microbiol. 5: 309–321). Natural infection of captive macropods by atypical mycobacteria generally produces an epithelioid cell response and granulomata formation (Peet et al., 1982, Aust. Vet. J. 58: 215; Mann et al., 1982, J. Am. Vet. Med. Assoc. 181: 1331–1333). The tissue changes seen in the two koalas studied here are consistent with a primary infection by *M. ulcerans*.

It appears likely that infections of *M. ulcerans* in koalas follow a pattern similar to that seen in man. Cutaneous ulceration is the major feature, but occasional progression to deeper tissues may occur. This progression may be more prevalent in cases with long-standing infections (Radford, 1975, op. cit.).

The source of the initial infection of the koalas' skin with *M. ulcerans* is probably a direct entry of the organism into cutaneous abrasions sustained during fights, or while tree climbing. The soil of the low-level island in this study is frequently waterlogged. Thus, *M. ulcerans* may enter abrasions if these are contaminated with surrounding soil or water that may harbor the organism.

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Transfer of *Listeria monocytogenes* Between Frogs

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Following the evidence that orally infected frogs shed detectable amounts of *Listeria monocytogenes* (Botzler et al., 1975, J. Wildl. Dis. 11: 277–279), we determined whether these bacteria could be transmitted between frogs.

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Twelve male leopard frogs (*Rana pipiens*) were captured at Whitmore Lake, about 24 km east of the Edwin S. George Reserve, Livingston County, Michigan, in October 1969, and stored at the Amphibian Facility of the University of Michigan (Nace, 1968, Bioscience 18: 767–775). At the time of this study in February 1970, the frogs were approximately 1–2 yr old.

Four frogs were placed into each of three 48 cm × 25 cm × 23 cm cages to