Transfer of Listeria monocytogenes Between Frogs

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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

which 550 ml of sterile tap water had been added. In contrast to the cages described by Nace (1968, op. cit.), the bottom component of our assembly was a solid acrylonitrile clear cage without siphon tubes or dry shelves. Thus, the frogs remained in the water throughout the experiment. Room temperature varied from 17–20 C. Water was not changed during the study.

Two strains of *Listeria monocytogenes* were used. Strain 2108 was isolated from a healthy white-tailed deer (*Odocoileus virginianus*) (McCrum et al., 1967, Bull. Wildl. Dis. Assoc. 3: 98–101). Strain F19-71 was isolated from a leopard frog (Botzler et al., 1973, J. Wildl. Dis. 9: 163–170). Both strains were pathogenic to laboratory mice; however, intraperitoneal injections of 10^8 listeriae of either strain into frogs resulted in no mortality in 10 days.

By use of polyethylene tubing inserted through the mouth into the stomach, two frogs of Cage A were each inoculated orally with 0.1 ml containing $10^{8.2}$ washed listeriae of Strain 2108; the other two frogs were not inoculated. Two frogs in Cage B each received 0.1 ml containing $10^{8.3}$ washed listeriae of Strain F19-71, while the other two frogs were not inoculated. None of the frogs in Cage C were inoculated. Individual frogs were marked by clipping their toes.

The number of listeriae in the water was monitored just before inoculation of the frogs and for 5 days following inoculation. Each day 10 ml were sampled from each cage after the water was mixed by gentle agitation. The sample was thoroughly mixed and counts of bacteria were estimated by serial dilutions on tryptose agar with 3.38% potassium thiocyanate (Botzler et al., 1973, op. cit.).

After 5 days, all animals were killed. Samples of liver, spleen, testes, and colon contents were incubated at 26 C in trypticase soy broth with 0.5% yeast extract. After 2 hr incubation, one ml of each sample was overlaid onto 9 ml tryptose broth with 3.75% potassium thiocyanate and incubated at 26 C. After 48 hr, a loopful of each broth was streaked for isolation on tryptose agar. Suspect colonies observed under oblique light were confirmed as *Listeria monocytogenes*.

The mean number of listeriae/ml of water in Cage A were: Day 0: 0; Day 1: 320; Day 2: 3,500; Day 3: 650; Day 4: 550; and Day 5: 900. The mean listeriae/ml in Cage B were: Day 0: 0; Day 1: 33,000; Day 2: 71,000; Day 3: 58,000; Day 4: 45,000; and Day 5: 20,000. In both cages, the peak populations of listeriae occurred on the second day after the frogs were inoculated. Correcting for the volumes of water, the peak number of listeriae in Cage A was 0.6%, and in Cage B was 9.8%, respectively, of the original inocula given to the frogs. No listeriae were detected in Cage C on any day.

At the time of necropsy, no abnormalities were seen in any of the organs from the frogs in Cages A, B, or C. Listeriae were isolated from the colon contents from both frogs of Cage A originally inoculated with Strain 2108, and from one of the two uninoculated animals; no listeriae were recorded from any other tissues. The same pattern was found among the frogs in Cage B: listeriae were isolated from the colon contents of both frogs originally inoculated and one of the two uninoculated animals; no listeriae were recovered from any other samples.

From these results, it is evident that *Listeria monocytogenes* can be passed between frogs. It is possible that the listeriae could have been transmitted by direct contact. However, the consistent occurrence of the bacteria in the water and the finding of the listeriae only in the intestinal tract of the uninoculated frogs suggest that ingestion of contaminated water was the most likely means of transmission.

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