

Aquatic Snails, Passive Hosts of *Mycobacterium ulcerans*

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Accumulative indirect evidence of the epidemiology of *Mycobacterium ulcerans* infections causing chronic skin ulcers (i.e., Buruli ulcer disease) suggests that the development of this pathogen and its transmission to humans are related predominantly to aquatic environments. We report that snails could transitorily harbor *M. ulcerans* without offering favorable conditions for its growth and replication. A novel intermediate link in the transmission chain of *M. ulcerans* becomes likely with predator aquatic insects in addition to phytophage insects. Water bugs, such as *Naucoris cimicoides*, a potential vector of *M. ulcerans*, were shown to be infected specifically by this bacterium after feeding on snails experimentally exposed to *M. ulcerans*.

Mycobacterium ulcerans is the causative agent of Buruli ulcer, one of the most common mycobacterial diseases of humans. This environmental mycobacterium has been found in swampy and wetland habitats that are home to aquatic insects implicated in the transmission of this pathogen (3–7, 10). Recently, Marsollier et al. found that certain aquatic macrophytes stimulated the growth of *M. ulcerans* and that the bacterium had a strong tendency to form biofilms on the plants' surfaces (2). In this report, we demonstrate the following results: (i) a few aquatic snails may harbor *M. ulcerans* after consuming aquatic macrophytes where an *M. ulcerans* biofilm has developed, (ii) no mycobacterial growth was detected in aquatic snails, so they may be a passive host, and (iii) after having eaten experimentally infected snails, the salivary glands of biting naucorid water bugs were found to contain *M. ulcerans*.

Experimental infection of snails. Tropical aquatic *Pomacea canaliculata* (Ampullariidae) and *Planorbis planorbis* (Planorbidae) snails 0.5 to 0.8 cm in total shell length were housed in an aquarium with water at a temperature of 28°C without aquatic vegetation and starved for 10 days. For a 24-h period, 30 snails were placed in additional aquaria containing aquatic vegetation covered by an *M. ulcerans* biofilm (strain number 1G897, isolated from a skin biopsy sample from a patient from French Guiana [1]) as previously described (2). The presence of an *M. ulcerans* biofilm on aquatic plants was confirmed by scanning electron microscopy (2). The number of acid-fast bacilli (AFB) on aquatic plants was evaluated by the method described by Shepard and Rae (9) at 10⁶ bacilli/g of plant tissue. After this period, the snails were removed and placed in

a noncontaminated aquarium without mycobacteria. At different intervals, four snails were sacrificed, the shells were removed, and the individual tissues were ground and homogenized. A PCR was performed for the detection of *M. ulcerans* as previously described (3, 8). The AFB were counted and cultured with the methods described by Marsollier et al. (3). During the 72 h after feeding on vegetation-bound *M. ulcerans* cells, viable bacilli were found in the feces of the snails. Within the first 10 days of the experiment, a slight but nonsignificant increase in AFB was observed, whereas the number of AFB in snail tissues remained steady for 30 days after feeding on vegetation-bound *M. ulcerans*. Then the number decreased gradually, until AFB were no longer detected after 50 days (Fig. 1). *M. ulcerans* was isolated by culture only at 1, 7, and 25 days postfeeding. Some snails were also infected by other bacteria or fungi. By PCR, *M. ulcerans* was detected 50 days postfeeding. Negative controls were checked with snails feeding on the same kind of vegetation free of *M. ulcerans*.

On examination of 5- μ m sections of sagittal tissue from infected snails, Ziehl-Neelsen staining showed that AFB were restricted to the digestive tract (Fig. 2). Twenty snails were placed in additional aquaria containing aquatic vegetation covered with *Mycobacterium kansasii* (strain number 11B0014, isolated from tap water) or with *Mycobacterium szulgai* (strain number 01CIMS02, isolated from an aquatic environment in Ivory Coast). Under our experimental conditions, no mycobacterial growth could be detected. Two months after the onset of experiments, no AFB were found. Moreover, AFB were rarely observed in the digestive tracts of control snails. All these data show that aquatic snails may be passive hosts of *M. ulcerans* and other environmental mycobacteria.

Infection of aquatic bugs. Adult biting water bugs, *Naucoris cimicoides* (Naucoridae), that were 2.5 cm long were collected from swamps in the west of France. The insects were main-

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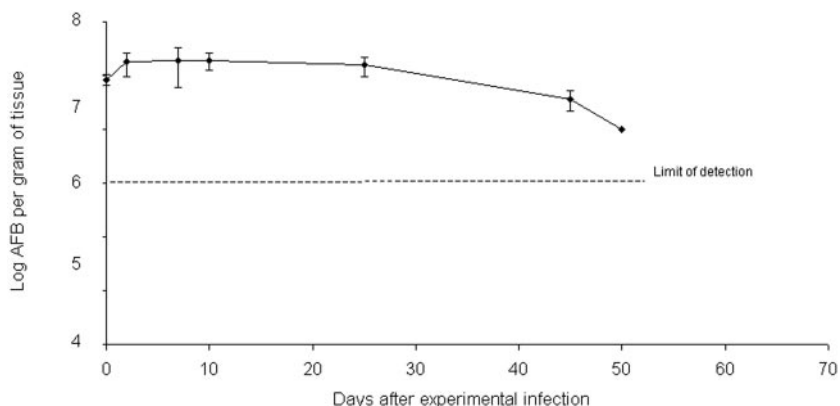


FIG. 1. Evolution of AFB in aquatic snail tissues (*Pomacea canaliculata*). The snails were experimentally infected after feeding for 24 h on aquatic vegetables covered by an *M. ulcerans* biofilm (day 0). Data are expressed as means ± standard deviations ($n = 4$).

tained in an *M. ulcerans*-free aquarium as previously described (3). Ten bugs were placed in an aquarium with 20 specimens of *Pomacea canaliculata* experimentally infected by *M. ulcerans*. The snails were the exclusive food for the carnivorous water bugs. After 7 weeks, *M. ulcerans* was detected both by PCR and by histological studies in two insects, with a bacterial distribution localized only in the salivary glands.

Aquatic snails from areas where *M. ulcerans* is endemic. Ten snails from the Planorbidae family (*Planorbis* sp. and *Bulinus* sp.) were collected in the Daloa region (Ivory Coast), an area known to be heavily affected by Buruli disease. Collections were made from the Lobo River in March 2001, just before the rainy season. By PCR, *M. ulcerans* was detected in one planorbid and one bullin. After decontamination (3), the samples were inoculated onto Löwenstein medium and incubated at

30°C without any successful isolation of the *M. ulcerans* strain. The samples also contained other mycobacteria, such as *Mycobacterium gordonae* and *M. szulgai*, or fungi. Furthermore, along the same river, *M. ulcerans* has been shown to be associated with aquatic bugs and vegetation (2, 3). Study of another set of snails collected in Papua New Guinea in July 2003 yielded similar observations in line with our hypothesis (data not shown). Recently, on the banks of the Oueme River in Benin, *M. ulcerans* was detected by PCR in 4% of the aquatic snails ($n = 86$) collected (unpublished results).

In tropical or nontropical regions, aquatic snails are the hosts of numerous organisms responsible for human infections, such as schistosomiasis and fascioliasis. These aquatic consumers may concentrate microorganisms, such as environmental mycobacteria and *M. ulcerans*, thereby participating in the dis-

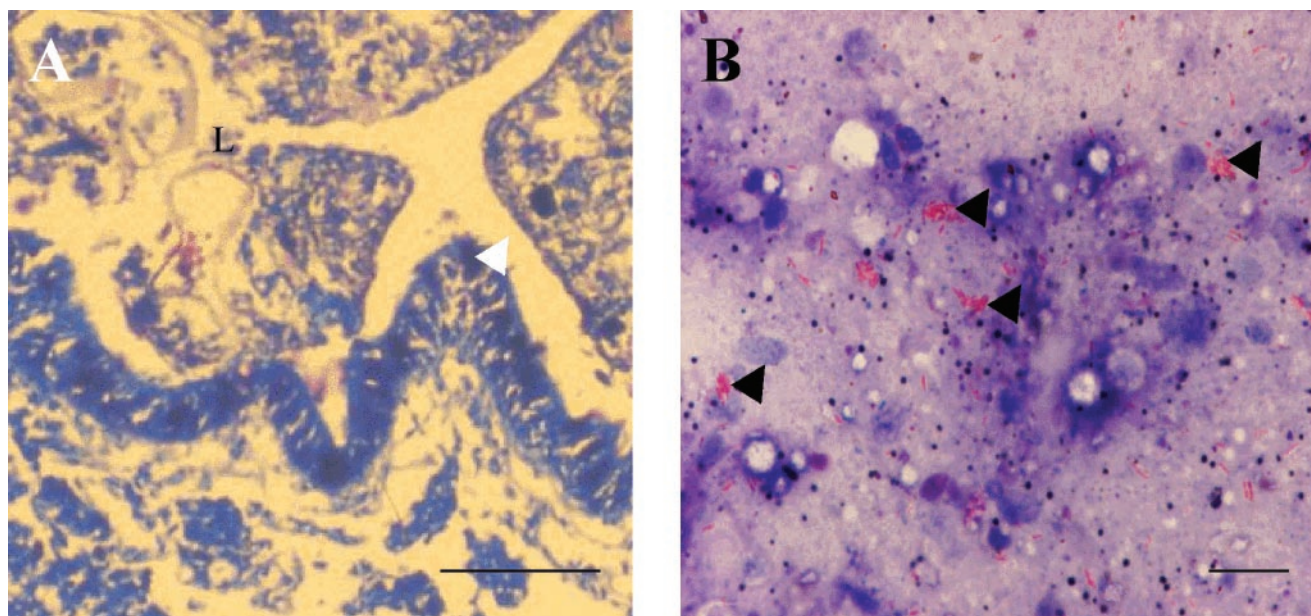


FIG. 2. Localization of *M. ulcerans* in 5- μ m sections of tissue from infected snails (*Planorbis planorbis*). (A) Ziehl-Neelsen-stained sagittal sections of an experimentally infected snail show a partial view of the lumen (L) of the digestive tract limited by epithelial cells (arrowhead) and filled by amorphous material (B) that at higher magnification contains aggregates of *M. ulcerans* cells (arrowheads). Scale bars: A, 200 μ m; B, 40 μ m.

semination of the bacilli in the environment. To test the possibility that other animals, such as the larvae of insects and possibly small fish, might also play a role in dissemination, we examined such potential vectors. Using PCR, we detected *M. ulcerans* in 6% of 60 larvae of dragonflies (of the families Gomphidae and Libellulidae) and in 70 juvenile fishes (of the family Clariidae) in Benin near the Oueme River (unpublished data). A network of aquatic agents begins gradually to be delineated in the spread of *M. ulcerans* infections. Further studies are needed to appreciate the role of the *M. ulcerans* virulence plasmid in the chain of transmission to humans and its behavior in certain environmental niches (11).

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